Molecular Recognition

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Imprinted Photonic Polymers for Chiral Recognition**

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Molecular imprinting is a well-established and facile technique to synthesize molecularly imprinted polymers (MIPs) with specific molecular-recognition sites for a large variety of applications, such as chromatographic separations, membranes, catalysis, biosensors, and artificial antibodies.[1–12] Molecular recognition and separation is generally based on bulk polymers, which have the disadvantages of long diffusion paths and the absence of accessible cavities for the analyte in the bulk, thus resulting in low specific capacities and long response times. Recently, the groups of Mosbach and Sellergren overcame these drawbacks of bulk MIPs by using hierarchical imprinting of template molecules on the surface of a porous silica support.[6–10] However, for assays with traditional MIPs, the analyte must contain a chromophore or fluorophore or be electroactive to generate a readable optical or electronic signal. Otherwise, the analyte must be modified or tagged. Thus, it is attractive to prepare label-free sensitive films[11–12] that give a readable signal upon binding guest molecules to detect and quantify the target analyte directly.

Colloidal crystals have been widely used as templates to create porous films with highly ordered 3D inverse opal structures.[13–15] The periodic variety of the refractive index of these porous films gives rise to interesting optical properties.[16–18] Particularly, if the films are made from hydrogel polymers, they may swell or contract in aqueous solution upon environmental changes, which leads to spectral shifts in the Bragg diffraction wavelength. Thus, they can be used as self-reporting sensors to measure various environmental changes, such as pH and temperature.[19–23] In this respect, Asher's group developed several hydrogel photonic films to sense Pb^{2+}, glucose, and creatinine.[19–20] Braun's group also synthesized hydrogel films of inverse opal structure that displayed a tunable optical response sensitive to pH value and glucose.[22–23] It was conceivable that, if two promising concepts (molecular imprinting and photonic crystals) were

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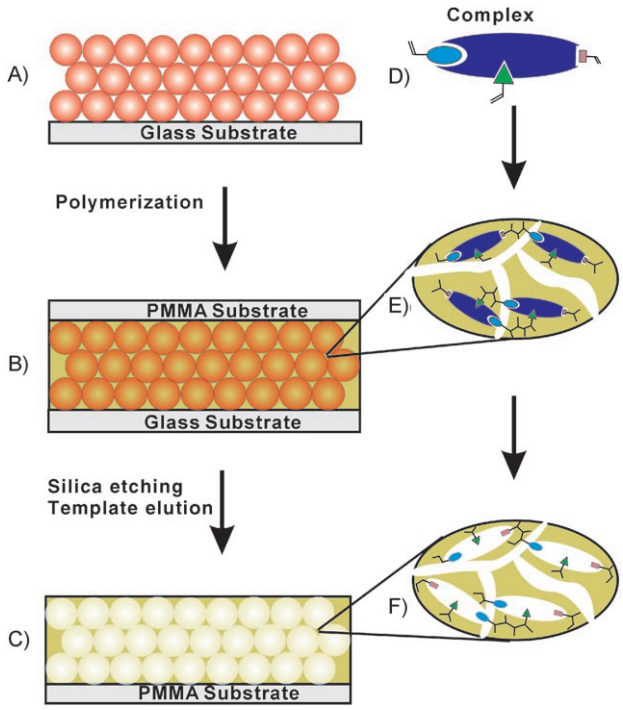
combined, a novel self-reporting sensing film could be constructed that exhibits high sensitivity and selectivity.

Herein, we adopt colloidal silica crystals as a template in collaboration with molecular-imprinting techniques to prepare highly ordered 3D macroporous hydrogel films that display highly selective and specific chiral molecular recognition properties. These films generate a readable optical signal directly (self-reporting) upon binding a target analyte without the need for labeling. Scheme 1 outlines the preparation of the inverse opal hydrogel films imprinted with chiral molecules. Monodisperse silica particles were fabricated by the Stöber method,[24] and a colloidal array template with highly ordered 3D structure was generated on a glass substrate by vertical deposition[25] of the silica spheres in ethanol (Figure 1 A). The size of monodisperse silica particles can be tuned in the range of 150 to 400 nm by changing the fabrication conditions. In the present work, monodisperse silica spheres with a diameter of 186 nm were used to form close-packed face-centered-cubic (fcc) photonic crystal films with a thickness of about 2 μm as a template (see the Supporting Information). Methacrylic acid (MAA) was used as functional monomer, and L-3,4-dihydroxyphenyalanine (L-dopa), a chiral electroactive analyte, was chosen as the template molecule in the molecular-imprinting process. Prior to its infiltration into the crystalline voids of the silica, MAA was mixed with L-dopa in a mixture of water and ethanol for sufficient intermolecular interaction to form a stable complex. Appropriate amounts of ethylene glycol dimethylacrylate (EGDMA) as cross-linker and 2,2′-azobisisobutyronitrile (AIBN) as initiator were added, and the mixture of precursors in the interstitial voids of the colloidal crystal template was photopolymerized in an ice bath. The silica nanoparticles were removed with 1% hydrofluoric acid, and L-dopa was eluted with 0.1M acetic acid. The film was rinsed with deionized water to obtain a macroporous L-dopa-imprinted hydrogel film (MIP film; Figure 1 B). These films with a thickness of about 2 μm (see the Supporting Information) not only have well-defined 3D ordered macroporous structure and large internal surface area, but also have a number of cavities left by the imprinted molecules, with complementary shape and binding sites[1–3] to L-dopa. Moreover, the macropores of these films are interconnected, which is favorable for the transport of molecules and significantly enhances the sensing response by shortening the diffusion path of analyte during the binding process.



Scheme 1. Protocol for the preparation of photonic MIP films: A) SiO_2 colloidal crystals on glass substrate; B) infiltration of complex solution into colloidal template followed by photopolymerizition; C) photonic MIP film after the removal of SiO_2 nanoparticles and dopa template molecules; D) complex of monomer and template molecule; E) imprinted molecules within the polymer matrix; F) imprinted cavities with complementary shape and binding sites to the template molecule.

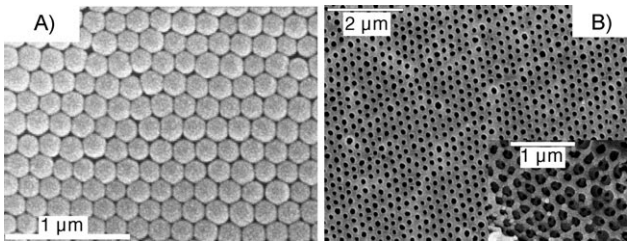


Figure 1. A) SEM image of colloidal crystal formed with SiO_2 spheres of diameter 186 nm. B) Top-view SEM image of the fabricated photonic MIP film. Inset: Magnified cross-section SEM image of MIP film showing the interconnection between the macropores of the fabricated MIP film.

L-Dopa is an amino acid that is able to reversibly form a stable complex with MAA by noncovalent interactions (e.g., electrostatic, hydrogen bonding, van der Waals).[5] These intermolecular interactions between L-dopa and MAA can be detected by fluorescence spectroscopy. Figure 2 A shows the fluorescent emission spectra of L-dopa in deionized water as well as in an MIP film. As a control experiment, the spectrum of a non-imprinted polymer film (non-MIP) prepared under the same conditions is also shown. A red shift of the emission peak from 320 to 331 nm is evident for L-dopa molecules

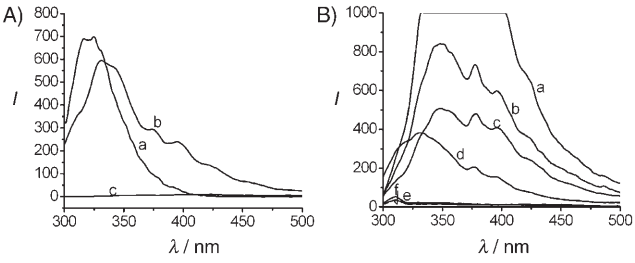


Figure 2. A) Fluorescence spectra of 1 mM L-dopa in deionized water (a), MIP film with entrapped L-dopa (b), and non-MIP film (c) on a PMMA substrate. B) Fluorescence spectra of MIP films exposed to L-dopa solutions of different concentrations.

trapped in the MIP film, but no emission is observed for the non-imprinted film. This result indicates that L-dopa was entrapped in the MIP matrix and has strong interaction with the polymer matrix, which leads to the shift of its emission peak. The imprinting of L-dopa in the polymer films was also confirmed by fluorescence spectroscopy. After the etching and eluting processes, the blank polymer films obtained were immersed into aqueous solutions of pure L-dopa or D-dopa at various concentrations. After thorough rinsing with deionized water to remove nonspecifically adsorbed analyte, the polymer films were checked by fluorescence spectroscopy for selective chiral adsorption. As expected, fluorescence was observed for the films exposed to L-dopa solution (Figure 2B). For the sake of comparison, the fluorescent spectra of the relative concentrations of pure L-dopa in solution are given in the Supporting Information. In agreement with the results in Figure 2A, there is a red shift of the emission peak when L-dopa molecule is rebound to the MIP film. Moreover, it was found that the extent of the red shift is dependent on the concentration of L-dopa in the solution (Figure 2B). When the concentration of L-dopa was 0.01 mM, a maximum red shift (28 nm) of the emission peak was observed. In contrast, no emission was observed for MIP films immersed in aqueous solutions of D-dopa at various concentrations. These results clearly indicate that the prepared MIP film has distinctive chiral selectivity for dopa. The imprinted cavities in polymer film with complementary shape and binding sites only to L-dopa are responsible for the observed chiral recognition. Also, the formation of polymer domains with lower dielectric constants possibly favors such interactions.

The chiral recognition of the prepared MIP films was also studied by UV/Vis spectroscopy by exposing these blank MIP films to a series of solutions of pure enantiomer of dopa at different concentrations. The MIP films were incubated in pure L-dopa or D-dopa in phosphate buffer solution (0.1M phosphate buffer) for 2 minutes, and their UV/Vis spectra were measured (Figure 3). The optical absorbance of the macroporous MIP film is very sensitive to the rebinding of L-dopa molecule, and the absorption peak shifts gradually to shorter wavelength (blue shift) with increase in the concentration of L-dopa (Figure 3A). The absorbance peak of an MIP film soaked in 10 mM L-dopa is blue shifted by 66 nm relative to that of the blank MIP film in 0.1M phosphate buffer. In contrast, the absorption peak remains almost unshifted for MIP films exposed to various concentrations of D-dopa (Figure 3B). Clearly, owing to its complementary shape with the binding sites, only L-dopa rather than D-dopa can occupy the imprinted cavities within the MIP films. Furthermore, as the isoelectric point of dopa is 5.52 at 25 °C,[26] the dopa molecules exist mainly in zwitterionic form in neutral buffer solution. The binding of L-dopa to MIP films is more effective through electrostatic interactions, which results in contraction of the hydrogel and a consequent blue shift of absorption peak of the MIP film. Notably, this enantioselective detection is highly sensitive, as evident from the detection of 0.01 μM dopa solutions (Figure 3A). Also, as a result of the interconnected macroporous structure, the prepared MIP films exhibit very fast response times, and the binding process can be completed within 20 s (see the

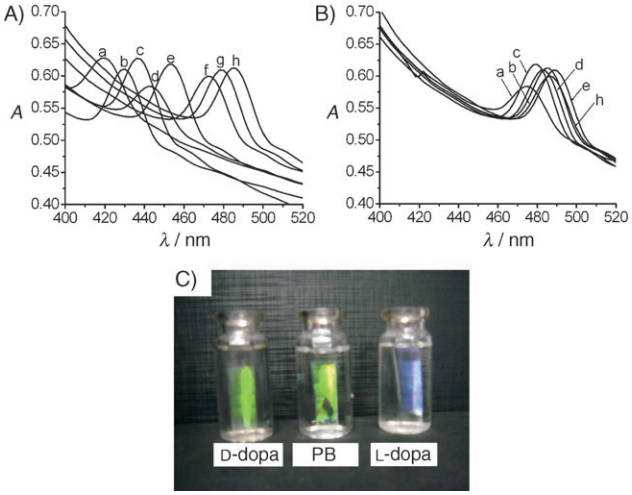


Figure 3. UV/Vis spectra of blank MIP films exposed to L-dopa (A) and D-dopa (B) in phosphate buffer at different concentrations: 10 mM (a); 1 mM (b); 0.1 mM (c); 0.01 mM (d); 1 μM (e); 0.1 μM (f); 0.01 μM (g), and deionized water (h). C) Three blank MIP films exposed to 0.1 M phosphate buffer (PB), 10 mM D-dopa, and 10 mM L-dopa in phosphate buffer.

Supporting Information). More importantly, the chiral recognition process can be easily and rapidly visualized by the naked eye (Figure 3C). Moreover, the binding behavior of L-dopa is affected by the pH value of the buffer solution. The rebinding efficiency decreases with decreasing pH value (see the Supporting Information). The neutral buffer solution provides an effective environment for the molecular recognition of the MIP film. The preparation of the MIP film as well as the characterization of its chiral recognition behavior was repeated several times, and good reproducibility was observed.

Consistent with the results described above, electrochemical methods further confirmed the chiral recognition behavior of the prepared MIP films. The experimental details as well as the results are presented in the Supporting Information.

The prepared MIP hydrogel films were evaluated under acidic and basic conditions. In particular, besides the chiral recognition function, these ordered macroporous hydrogel films can swell or contract upon changes of certain external conditions, such as pH value, temperature, ionic strength, and added electrolyte.[19,22] Figure 4 shows that upon changing the solution from 0.1M HCl to 0.1M NaOH, the absorption peak of the film is red shifted, which is in agreement results reported by Lee and Braun.[22] The reason for this shift is the presence of carboxyl groups with the macroporous network which can be deprotonated when OH^- ions diffuse from the bulk solution into the gel phase. As a result, the osmotic pressure within the gel is increased owing to the increased Donnan potential inside the polyelectrolyte network; this pressure increase drives the hydrogel swelling.

In summary, to the best of our knowledge, this is the first report of a general protocol for the synthesis of an ordered 3D macroporous MIP film with a self-reporting chiral recognition function by using both colloidal crystals and molecular-

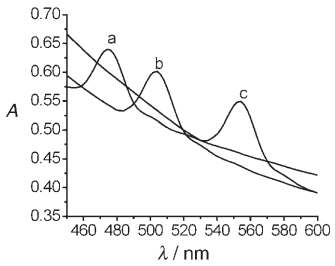


Figure 4. Normal incidence transmission UV/Vis spectra of the prepared MIP film exposed to 0.1 M HCl (a), deionized water (b), and 0.1 M NaOH (c).

imprinting techniques. The current method emphasizes the use of a readable optical signal caused by structural changes upon binding of the analyte. The result shows that this special MIP film has many advantages in specific recognition, such as quick response time, high sensitivity, and high selectivity. The described concept not only provides a potential alternative to create optical diffraction-based chemical or biological sensors, but also extends the applications of MIP films in other areas such as drug separation, clinical assays, and catalysis.

Experimental Section

Monodispersed silica particles were synthesized by the Stöber method. Colloidal SiO_2 crystals were fabricated by vertical deposition on glass slides. These preparations are described in the Supporting Information. MAA (4 mL, 47.16 mmol, Acros), EGDMA (2 mL, 10.59 mmol, Acros), and L-dopa (0.02 g, 10.14 mmol, Sigma) were mixed in a mixture of anhydrous ethanol (3 mL) and deionized water (1 mL). AIBN (ca. 0.01 g, 6.1 mmol, Acros) was added, the mixture was bubbled with nitrogen for 10 minutes, and the resulting homogeneous monomer mixture was infiltrated into the colloidal silica crystals. Once the colloidal crystals became transparent, excess precursors were removed by covering a clean PMMA slide (1.5 mm thick), and the remaining mixture was photopolymerized in an ice bath under a UV light at 365 nm (16 W) for 2 h. The sandwich structure was immersed in 1 % HF solution for 2 h to separate double slides and completely etch the silica colloids. The formed polymer was washed with 0.1M acetic acid for 2 h and then rinsed thoroughly with deionized water until no L-dopa was detected by fluorescence at 320 nm in the eluent, thus indicating full removal of L-dopa from the imprinted films. For preparation of free-standing films, a glass slide was used instead of the PMMA slide as the cover.

The fluorescence spectra of pure L-dopa in deionized water and L-dopa entrapped in the MIP film were obtained with a Perkin–Elmer LS55 spectrometer. The blank MIP films obtained by complete removal of the L-dopa template were used for the rebinding process. An excitation wavelength of 282 nm was used for the fluorescence measurements, and the slit width was 8 nm. All optical responses the of MIP films supported on PMMA were performed on an UV/Vis spectrometer (Perkin–Elmer Lambda 35). Phosphate buffer (0.1M, pH 7.6) was prepared from NaH_2PO_4 and Na_2HPO_4 (Beijing Chemicals), and various D- and L-dopa solutions were prepared at various concentrations in the phosphate buffer. In all cases, prior to detection, the MIP films were washed with 0.1M acetic acid followed by deionized water until no dopa was detected. After each UV/Vis measurement, the film was rinsed with deionized water and phosphate buffer. The sequence of measurements was performed from low to high concentration to eliminate contamination.

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