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Electronic Supplementary Information

Molecular Imprinting in Pickering Emulsions: New Insight into Molecular Recognition in Water

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1. Materials

Silica nanoparticles (diameter 10 nm), ethylene glycol dimethacrylate (EGDMA, 98%), atenolol (98%), metopronolol (+)-tartrate (97%), timolol maleate (98%), pindolol (98%) and 1-naphthol were purchased from Sigma-Aldrich (Gillingham, UK). Methacrylic acid (MAA, 98.5%), azobisisobutyronitrile (AIBN, 98%), and Triton X-100 (99.5%) were obtained from ACROS (Geel, Belgium) and Merck (Darmstadt, Germany). (*R,S*)-Propranolol hydrochloride (99%) and acriflavin (AFN, ≥ 90%) were supplied by Fluka (Dorset, UK). [^{3}H]-(*S*)-Propranolol (specific activity 555 GBq mmol^{-1}, 66.7 μM in ethanol solution) was purchased from NEN Life Science Products, Inc. (Boston, MA). Scintillation liquid, Ecoscint A was purchased from National Diagnostics (Atlanta, GA). AIBN was recrystalized from methanol. Other solvents and inorganic salts were of analytical reagent grade and were used without further purification.

2. Preparation of MIP by Pickering emulsion polymerization

Silica nanoparticles (20 mg), MAA (0.272 mL), NaOH solution (0.5 mL, 3 mol L^{-1}) and Triton X-100 solution (6 mL, 0.3%) were mixed and sonicated for 10 min. After addition of EGDMA (1.728 mL) containing template (60 mg of propranolol), porogen (200 uL of toluene) and initiator (20 mg of AIBN), the mixture was again sonicated for 10 min and shaken vigorously for ~ 1 min by hand. A stable Pickering emulsion was obtained when no coalescence of the oil droplets could be observed within 2 h (Note: Without the silica nanoparticles, Triton X-100 itself did not give stable emulsions). After polymerization (without agitation) at 70 °C for 16 h, composite polymer/silica particles were obtained. After the reaction product was kept at room temperature for 1 h, the supernatant in the mixture was removed. To remove the soluble polymers, the particles were added to 5 mL tetrahydrofuran (THF) and shaken vigorously. This step was repeated one more time. After decantation, the solid particles were washed with methanol and water. To remove the silica nanoparticles, the composite microspheres were transferred into a plastic tube and stirred in 4 mL HF (30%) at room temperature for 12 h. Following this step, the solid polymer microspheres were washed with methanol containing 10% acetic acid, until no template could be detected from the washing solvent busing UV spectrometric measurement. The polymer particles were finally washed with methanol and

dried in a vacuum chamber. As a control, non-imprinted microspheres were prepared following the same procedure, except that no template was added.

3. Preparation of MIP by bulk polymerization

An oil phase containing monomer (0.272 mL of MAA), cross-linker (1.728 mL of EGDMA), porogen (200 uL of toluene), template (60 mg of propranolol) and initiator (20 mg of AIBN) was first prepared. The oil phase was mixed with 6 mL of water and shaken vigorously for 3 min. After a brief centrifugation, the oil phase was separated. This water-saturated oil phase was then transferred to a glass tube and purged with nitrogen for 5 min. After polymerization at 70 °C for 16 h, the polymer monolith obtained was crushed and ground into fine particles using a mechanical ball mill. The polymer particles were washed with methanol containing 10% acetic acid, until no template could be detected from the washing solvent busing UV spectrometric measurement. The polymer particles were finally washed with methanol and dried in a vacuum chamber. A non-imprinted polymer (NIP) was prepared following the same procedure except that no template was added. The MIP and NIP prepared by bulk polymerization were named as MIP-B and NIP-B, respectively.

4. Fluorescent labeling with AFN

The carboxyl groups on the MIP or NIP particles were labelled with fluorescent acriflavin (AFN).[1] Briefly, 10 mg of imprinted particles were added into 1 mL of AFN solution (100 mg L^{-1} in methanol), and the mixture was stirred at room temperature in dark for 12 h. The particles were separated by centrifugation, and washed with methanol until no fluorescence could be observed in the supernatant. The particles were finally dried in a vacuum chamber. These AFN-labelled microspheres were deposited on a glass slide and observed with a Nikon Eclipse E400 epifluorescence microscope equipped with a CCD camera.

Fig. S1 is the fluorescence microscope image of AFN-labelled MIP-B particles. The fluorescence intensity of the particles is much lower than that observed on MIP-P particles (Figure 1c), indicating the density of carboxyl groups on MIP-B is much lower than that on MIP-P.

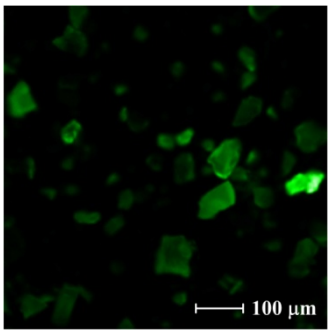

Fig. S1 Fluorescence microscope image of AFN-labelled MIP-B particles.

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5. Characterization of the imprinted particles

To illustrate the wetting properties of the imprinted particles, MIP particles were deposited on a flat surface and pressed into a thin layer using a watch glass. The wetting properties of the polymers were estimated by direct image analysis of the contact angle of water droplet (50 μL) on the polymer layers (**Fig. S2**). Assuming the roughness of the particle films are the same, the contact angle of water on MIP-P (< 90°C) is much smaller than that on MIP-B (> 90°C), suggesting that MIP-P is hydrophilic and MIP-B hydrophobic.

Fig. S2 Images of water droplets deposited on a thin layer of MIP-P (a) and MIP-B (b).

The surface structures of the imprinted polymers were studied by measuring the attenuated total reflection (ATR) infrared spectra on a Perkin-Elmer FTIR instrument (Perkin-Elmer Instruments). Approximately 2 mg of dry particles were placed onto the sample plate of the instrument, and the spectra in the 4000-375 cm^{-1} region were recorded with a resolution of 4 cm^{-1}, using 24 scans at room temperature.

The size and surface morphologies of the imprinted microspheres were observed with a scanning electron microscope (SEM; JEOL JSM-T300). **Fig. S3** are the SEM images of MIP-P and NIP-P microspheres. It can be seen that MIP-P and NIP-P have approximately the same particle size in the range of 100~200 μm.

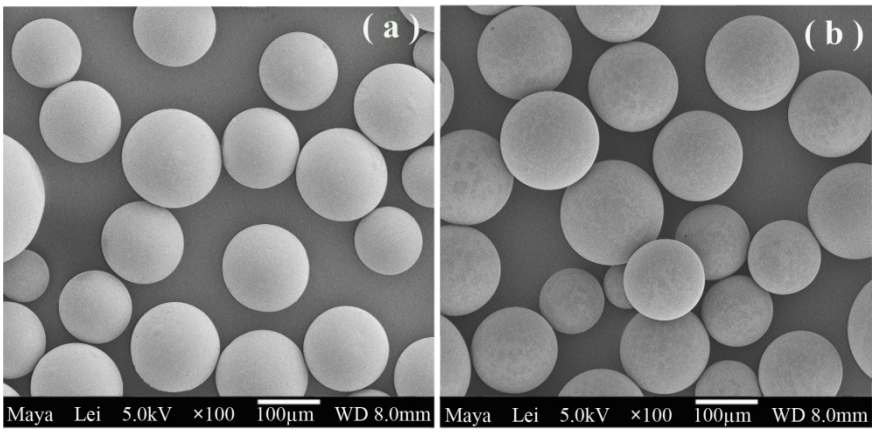

Fig. S3 SEM images of MIP-P (a) and NIP-P (b).

6. Radioligand binding analysis

Polymer particles (5 mg) were added into l mL of [^{3}H]-(*S*)-propranolol solutions (246 pmol L^{-1}). In displacement experiment, the competing compound was also added with an initial concentration of 3.38 μmol L^{-1}. The mixture was gently stirred at room temperature for 12 h. After centrifugation, 500 μL of supernatant was collected and added into scintillation liquid (Ecoscint A, 10 mL), and the radioactivity was measured with a Tri-Carb 2800TR Liquid Scintillation Analyzer (Perkin Elmer). The difference between the free radioligand and the total radioligand added was used to calculate the percentage of binding.

7. Fluorescence measurement

The solutions of propranolol in free base form were measured on a QuantaMaster C-60/2000 spectrofluorometer. The emission spectra of propranolol-loaded dry particles were measured using the same spectrofluorometer, where the dry particles (~ 5 mg) were embedded into a filter paper and exposed to the excitation beam with an angle of 45°. The excitation wavelength was set at 230 nm.

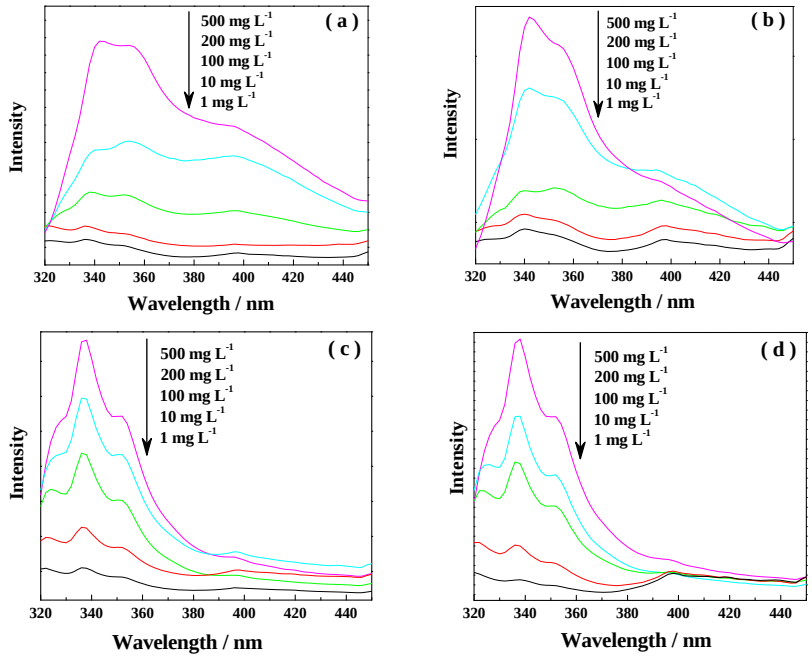

Fig. S4 Fluorescence excitation spectra of MIP-P (a), NIP-P (b), MIP-B (c), and NIP-B (d) with different concentrations of propranolol.

8. Swelling experiment

The swelling behaviours of the MIP microspheres were measured in water and toluene. Breifly, 10 mg of dry MIP microspheres and 1 mL of water (or toluene) were placed in a 2-mL microcentrifuge tube, and shaken vigorously for 3 min. The microcentrifuge tube was gently shaken for 12 h at 20 °C, before the excess water (or toluene) was removed by centrifugation and blotting with filter paper. The amount of solvent adsorbed by the MIP microspheres ($V_{solvent}$) was calculated using the following equation:

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$$V_{\text{solvent}} = \frac{m_s - m_0}{m_0 \times d}$$

where m_0 is the mass of dry microspheres, m_s is the mass of swollen microspheres, and d is the density of water (or toluene) at 20 °C. The amounts of solvent adsorbed by the MIP microspheres are shown in Table S1.

Table S1 Amount of solvent adsorbed by the MIP microspheres.

Synthetic method	V_{water} (mL g^{-1})	V_{toluene} (mL g^{-1})
Pickering emulsion	0.66	0.15
Bulk	0.47	0.96

Reference

1. J. A. A. Sales, A. G. S. Prado and C. Airoldi, *Polyhedron,* 2002, **21**, 2647.