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Electronic Supplementary Information

Triple-function zwitterion for preparing water compatible diclofenac imprinted polymers

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1. Materials

Diclofenac sodium, 2,2'-diamio-N-methyldiethylamine were purchased from TCI Chemical Ltd. (Tokyo, Japan). Acryloyl chloride (95%), 1,3-propane sultone (98%), 2-vinylpyridine (2-VP), N,N,N',N,'-tetramethylenediamine (TEMED, 95%), and mefenamic acid (MAC) were purchased from Beijing J&K Co., Ltd. (Beijing, China). N,N'-methylenelisacrylamide (MBA), ibuprofen sodium (IBP), sodium benzoate(SB), ammonium persulfate (APS), bovine serum albumin (BSA, M_w=68KDa, 90%), and fluorescein isothiocyanate (FITC) were all purchased from Sigma-Aldrich (Saint Louis, Missouri). BSA was dissolved in phosphate buffer (pH=7.4) with deionized water when used.

2. Characterization

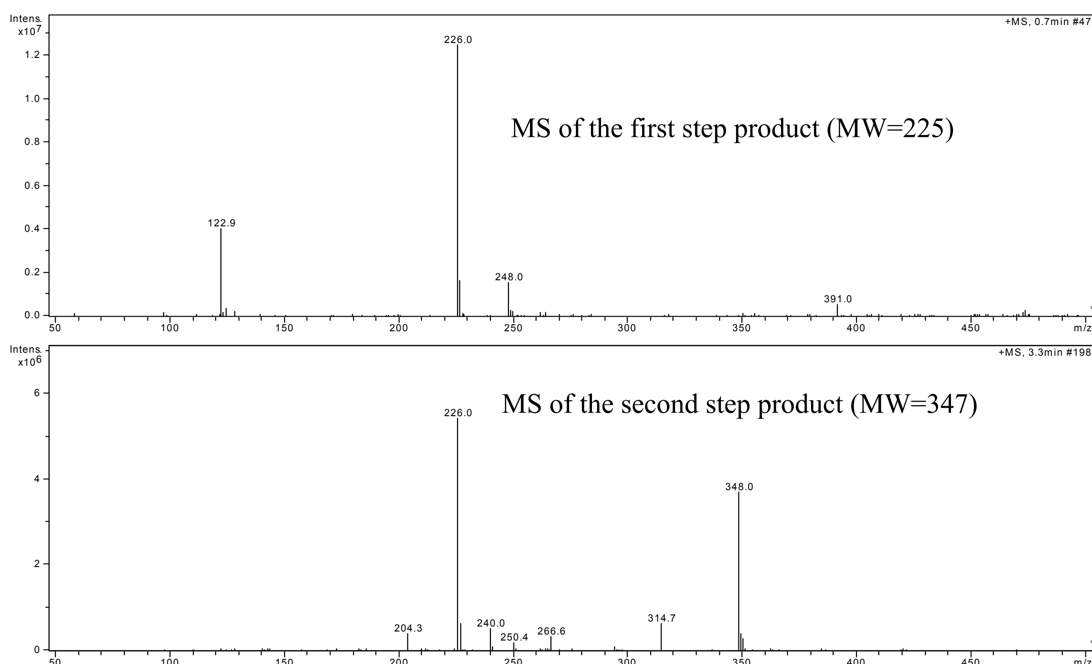
^{1}H NMR and ^{13}C NMR spectra (300 and 75 MHz for ^{1}H and ^{13}C, respectively) were recorded at room temperature in D_2O on BRUKER DPX (Karlsruhe, Germany). Surface morphologies were examined by SEM using a JEOL JSM 5400 scanning microscope from JEOL Ltd. Co. (Tokyo, Japan) at an accelerating voltage of 15 kV. Samples were mounted on metal stubs and coated with gold–palladium by Denton Vacuum Desc II. MS spectra were recorded using a JEOL JMS-700 mass spectrometer. The surface areas and porosities of the MIPs and NIPs were measured by nitrogen adsorption porosimetry using a TriStar surface area and porosity analyzer

(Micrometrics Instruments, Norcross, GA, USA). Prior to measurement, 200 mg samples of the polymers were heated at 80 °C for 5 h in vacuum. The specific surface areas were calculated using the BET method, and the specific pore volumes and average pore diameters were calculated by the BJH method.

3. Synthesis of AMAS

2,2'-diamio-N-methyldiethylamine (2.34 g, 0.02 mol) was dissolved in 25 mL of dichloromethane, and 7.33 mL sodium hydroxide solution (6 mol/L) was added. After that acryloyl chloride (3.80 g, 0.042 mol) was added dropwisely to the mixture under vigorous magnetic stirring at 0 °C. The mixture was reacted for 3 h, and the organic phase was subsequently washed twice with saturated sodium carbonate aqueous solution. After that, the dichloromethane was evaporated and first step product N,N-biacrylamidoethyl-methylamine was obtained in 85% yield.

N,N-biacrylamidoethyl-methylamine(2.25 g, 0.01 mol) was dissolved in 10 mL acetonitrile and hated to 40 °C. Then 3.66 g (0.03 mol) 1,3-propane sultone was dropped into the reactor within 0.5 h under vigorous magnetic stirring. The mixture was stirred at 40 °C for 12 h. The resulting white precipitate was filtered, washed with acetonitrile and acetone, and dried under vacuum at room temperature for 12 h. The white monomer (AMAS) was obtained in 80% yield. ^{1}H, ^{13}C, and MS were shown in Fig. S1.



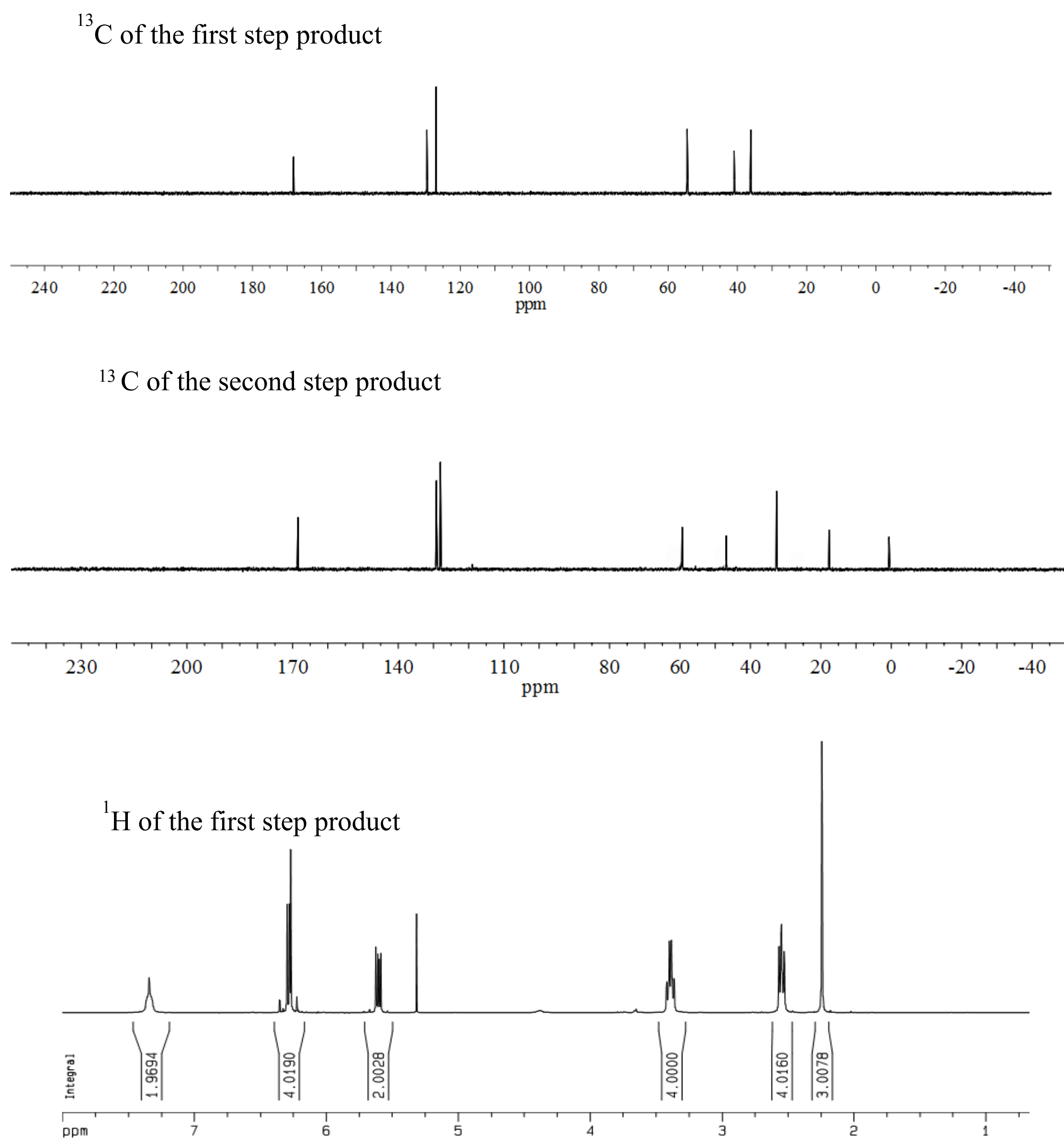


Fig.S1 MS, ^{13}C, and ^{1}H-NMR spectra of the first and second step product

4. Preparation of DFC-MIP

For the preparation of DFC-MIP, the template DFC (0.06 g, 0.2 mmol) and monomer AMAS (0.278 g, 0.8 mmol) were dissolved in 40 mL distilled water. After stirring for 1.0 h at 60 °C, 0.493 g (3.2 mmol) MBA and initiator APS (30 mg) and

TEMED (30μL) was added to the solution. The mixture was purged with nitrogen for 5 min to remove oxygen, and sealed in the glass vial. The temperature was ramped from room temperature to 60 °C over a period of around 2 h and then held at this temperature for a further 24 h. The resultant microspheres were then sonicated in methanol/acetic acid solution (9:1, v:v) for 20 min, followed by centrifugation to remove solvent. This procedure was repeated several times until DFC could not be detected in the extraction solvent. Then, the microspheres were again sonicated in methanol for three cycles of 15 min each to remove residual acetic acid. Finally, the solvent was removed by centrifugation and the microspheres were dried under vacuum. As a reference, non-imprinted polymer (NIP), which did not contain the template, was also prepared in parallel with the MIP by using the same synthetic protocol.

The content of AMAS as well as methylenebisacrylamide (MBA) in the MIPs was optimized. As shown in Fig.S2 (a), the amount of DFC adsorbed by 0% AMAS based MIPs (19 mg g^{-1}) was as low as corresponding NIPs (16 mg g^{-1}). However, the capacity jumped to 35 mg g^{-1} when 5% AMAS was used, and kept increasing as the molar ratios of AMAS increased. When 20% AMAS was added, the adsorption capacity of the MIP reached 77 mg g^{-1}. While more AMAS was added, the capacity of MIP increased very little and the adsorption capacity of NIP increased largely. Lower molar ratios induce less binding sites in polymers due to fewer template–monomer complexes, but over-high ones produce higher non-specific binding capacity, diminishing the binding selectivity. So, in this work, 20% of AMAS was used for the preparation of DFC imprinted polymers. The role of the cross-linker is to fix functional groups of functional monomers around imprinted molecules. In this work, the amount of MBA added in the MIP was investigated. As shown in Fig.S2 (b), the adsorption capacities of both MIP and NIP decreased as the crosslinking density increased. However, the maximum ratio of Q_{MIP}/Q_{NIP} reached when 3.2 mmol of MBA was used. This is because when the crosslinking density is too low, MIPs cannot maintain stable cavity configurations. However, over-high amounts of cross-linkers will reduce the number of recognition sites per unit mass of MIPs. Therefore,

3.2 mmol of MBA was used in this work.

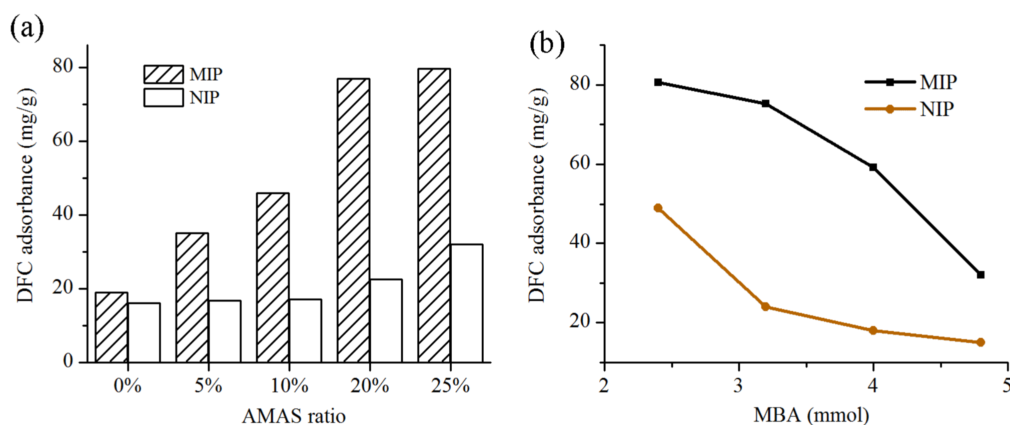


Fig.S2 Effect of AMAS (a) and MBA (b) on the adsorption capacity of MIP and NIP. The initial concentration of DFC was 100 mg/L.

5. Scanning electron microscope (SEM) and porosity measurements

As shown in Fig.S3, The NIP was observed to have smoother surface than the MIP, while the MIP after the template removal had rough surfaces which can be attributed to the formation of cavities during the synthesis process. Specific surface areas and pore volume of the imprinted polymers (48.90 m^2 g^{-1} and 0.10 cm^3 g^{-1}) was considerably higher than those of the non-imprinted polymers (19.679 m^2 g^{-1} and 0.067 cm^3 g^{-1}). These data demonstrated that the MIPs have many imprinted cavities created by removal of the template molecules. These cavities remembered the shape of the template and could be used to recognize the target template.

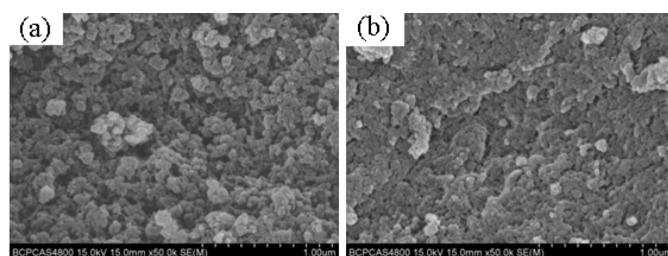


Fig.S3 SEM images of (a) MIP-AMAS and (b) NIP-AMAS

6. Binding characteristics of MIP

For the binding kinetics experiments, 10 mg of MIP-ZI and NIP-ZI was suspended in 5 mL diclofenac solution with the initial concentration of 1.0 g/L separately. Then the solution was shaken for 2 h at room temperature. To evaluate the binding isotherms capacity of the material, 10 mg of polymer microspheres was added

to a 15 mL centrifuge tube containing 5.0 mL diclofenac solution of various concentrations (200-1000 mg/L). After shaken for 2 h at room temperature, the samples were centrifuged and filtered. The concentration of diclofenac in the solvent phase was determined by UV- spectrophotometry. The adsorption capacity (Q) was calculated by subtracting of the free fraction from the initial concentrations using the following formula:

$$Q = (C_i - C_f) \times V / W$$

Where C_i (mg mL^{-1}) is the initial concentration of the analytes, C_f (mg mL^{-1}) is the final concentration of the analytes, V (mL) is the volume of the adsorption mixture, and W is the mass of polymer in each rebinding mixture.

Scatchard analysis was carried out by the equation $Q/C_{[DFC]}=(Q_{max}-Q)/ K_D$, where Q is the amount of DFC bound to MIPs at equilibrium, Q_{max} is the maximum binding capacity, $C_{[DFC]}$ is the free DFC concentration at equilibrium and K_D is the equilibrium dissociation constant of binding sites. The dissociation constant K_D of AMAS-MIP and NIP was calculated to be 2.16 mg/L and 251 mg/L, respectively. Compared to MIP for DFC reported in the literature (K_D=16.5 mg/L, Sun et al., Analytica Chimica Acta 620 (2008) 73-81), the K_D of AMAS-MIP in this study was much lower indicating stronger binding affinity.

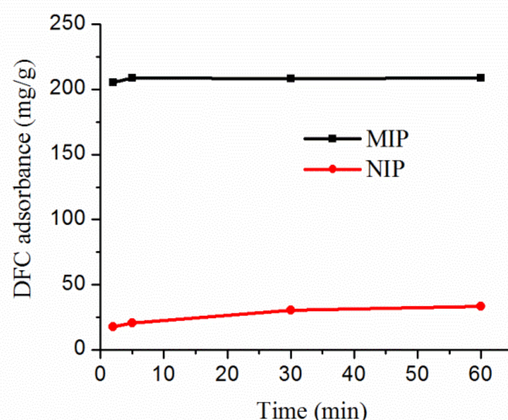


Fig.S4 dynamic adsorption curves of the MIPs and NIPs for DFC.

7. Specific recognition experiments of MIPs and NIPs with DFC in the presence of MAC, SB and IBP.

The MIPs and NIPs (10 mg in each case) were suspended in 5 mL of mixtures (DFC, MAC, SB and IBP, the concentration of each was 0.5 mg L^{-1}). The mixtures were agitated on a rocking table at ambient temperature for 30 min. The concentrations of different analytes in the supernatant were measured by HPLC. HPLC analysis was performed on a liquid chromatograph (Agilent 1260 Infinity) equipped with a vacuum degasser, quaternary pump (G1311B), and UV detector connected to a C18 column. The column temperature was 25 °C, and the mobile phase was methanol/acetate buffer (pH 3.8, 2:2 v/v) with a flow rate at 0.8 mL min^{-1} at the wavelength of 276 nm.

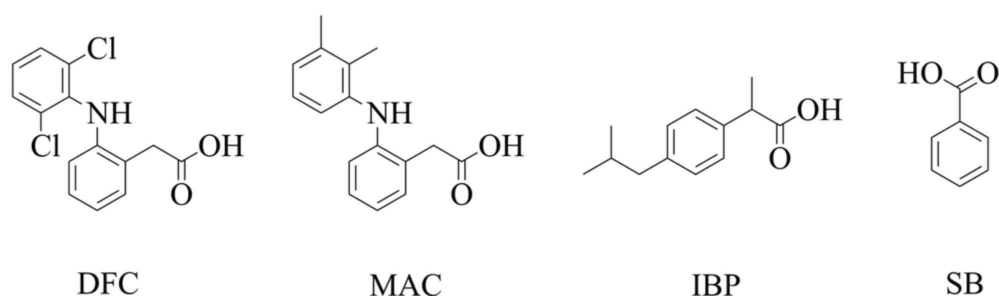


Fig.S5 Molecular structures of DFC, MAC, IBP and SB.

8. Resisting nonspecific protein adsorption

8.1. Fluorescent labeling with FITC

To visually reveal the function of AMAS resisting protein adsorption, BSA was labelled by fluorescein isothiocyanate (FITC). 10 mg BSA were added to 2.0 mL aqueous solution of FITC (1 mg/mL), and the mixture was incubated at 4 ℃ in dark for 8 h. To remove the excessive FITC, the proteins were washed with water and separated by centrifugation until no fluorescence could be observed in the flow through. The FITC-labeled proteins were stored at 4 ℃.

8.2. Fluorescence microscope observation and fluorescence spectrum of FITC-BSA

FITC was used to attach a fluorescent label on BSA via the amine group. The isothiocyanate group was reacted with amino terminal and primary amines in proteins. 10 mg of MIP or NIP was added in 1 mL of FITC-BSA (0.4g/L). After centrifuged three times to wash unabsorbed FITC-BSA, the solid particles were observed under fluorescence microscope (Olympus FV-1000). And the concentration of supernatant

was analyzed by fluorescence spectrum (Hitachi, F-7000).

8.3. Recovery of DFC from 10 mg/mL BSA samples

To a 2.0 mL tube, 10 mg of MIPs were added to 1 mL of BSA aqueous solution (10 mg/mL). Then, DFC at three different levels 10, 30, and 60 mg L^{-1} was added, respectively. The mixtures were agitated on a rocking table at ambient temperature for 30 min. Then the mixtures were filtered through 0.4 μm organic system filter membranes. The concentrations of DFC in the supernatant were measured by HPLC. The experimental result was shown in Table S1,

Table S1 The recovery of DFC in 10 mg/mL BSA samples

The amount of DFC (mg/L)			Recovery (%)
Added	Found		
	Mean[a]	R.S.D (%)[a]	
10.0	9.5	3.1	95
30.0	27.6	4.0	92
60.0	54.6	3.5	91

[a]: Average of three determinations; [b]: Relative standard deviation.