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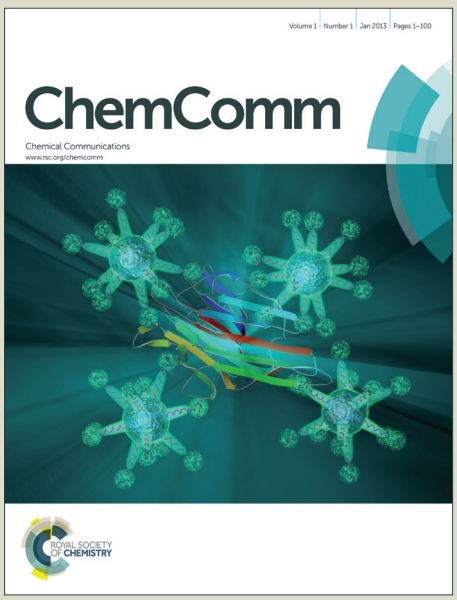

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Triple-function zwitterion for preparing water compatible diclofenac imprinted polymers

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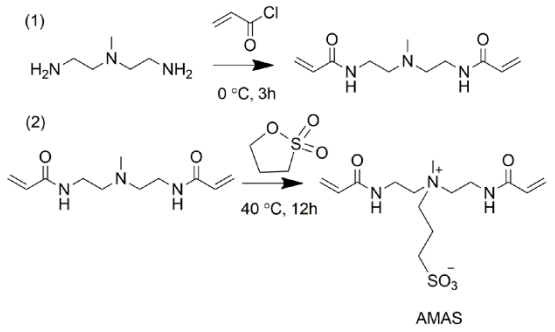
A novel zwitterion acting as both functional monomer and crosslinker, with protein-resistant ability concomitantly was synthesized for preparing water compatible diclofenac imprinted polymers. This new imprinted polymer showed high imprinting efficiency for template and strong anti-protein adsorption in aqueous medium.

Molecular imprinting technology (MIT) is a versatile and straightforward method to prepare artificial receptors with a predetermined selectivity and specificity for a given target molecule.[1-5] During the last decades, molecularly imprinted polymers (MIPs) have been extensively applied in many fields such as solid-phase extractions,[6, 7] chromatographic separations,[8] catalysis,[9, 10] drug delivery systems[11] and sensors.[12]

Despite the tremendous progress made in these fields, many challenges still remain to be addressed. Water incompatibility is the primary concern of imprinting technology.[13-16] The reported MIPs were normally prepared in organic solvent and showed poor specific binding for the template in pure aqueous media. This is because the presence of polar solvent, especially water, can disturb the hydrogen bond formation between template and functional monomer. Although hydrophilic modifications (e.g. HEMA[13], β-CDs[17]) of MIPs surfaces were employed to solve this problem, the processes were complex and time-consuming. Moreover, a large amount of cross-linking agent (around 80–90%) had to be used to preserve the memory of template in MIPs. As a result, only a small portion of the imprinted sites retained available leading to the undesired low adsorption capacities.[18-20] In addition to the problems mentioned above, biomacromolecules such as proteins always interfered with the determination of small target compounds in environmental water and biological samples. The nonspecific protein adsorption severely affected the sensitivity and capacity of MIPs for target template. A layer of porous hydrophilic polymer (e.g. chitosan[21]) coating on the surface of adsorption materials was explored to solve this problem, and the pore size after coating was appropriate to hold back the macromolecules without influencing the adsorption of target analytes. Poly(ethylene glycol) (PEG) is one of the best synthetic materials being widely used to resist nonspecific protein adsorption.[22] However, PEG is susceptible to decomposition in the presence of oxygen and transition metal ions.[23, 24] Recent studies have demonstrated that zwitterionic groups, including phosphorycholine, sulfobetaine, and carboxybetaine, have been widely used as new generation of non-biofouling materials which can strongly resist nonspecific protein adsorption via a hydration layer bound through solvation of the charged terminal groups in addition to hydrogen bonding.[23, 25-27] In 2010, Wang et al. reported that the addition of 2-methacryloyloxyethyl phosphorylcholine, a common zwitterion-type monomer, can potentially suppress the inflammation response of the MIPs biosensors.[28] So far, reports on zwitterion applied in MIP for resistance of non-specific protein adsorption are still few.



Scheme 1 Synthesis of AMAS

In this study, a novel kind of zwitterionic monomer, 3-(bis(2-acrylamidoethyl) (methyl) ammonio) propane-1-sulfonate (AMAS), was synthesized for preparing water compatible MIPs with strong resistance to non-specific protein adsorption. AMAS was synthesized according to Scheme 1. The newly prepared monomer bears zwitterionic groups in the middle together with two terminal vinyl groups on both sides. Zwitterionic groups provide the monomer with high hydrophilicity and electrostatic interaction with target template in aqueous media, which could be applied in preparing water compatible MIPs. The as-prepared MIPs can be expected to resist nonspecific protein adsorption due to the zwitterionic groups as well. Moreover, the new functional monomer AMAS could act as crosslinker simultaneously by virtue of the two terminal vinyl groups. Overall, the featured structure with two

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double bonds and zwitterionic groups could endow this new monomer with triple functions: functional monomer providing electrostatic interaction with target template, water soluble crosslinker, and strong resistance to protein adsorption.

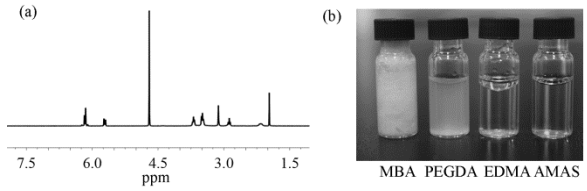
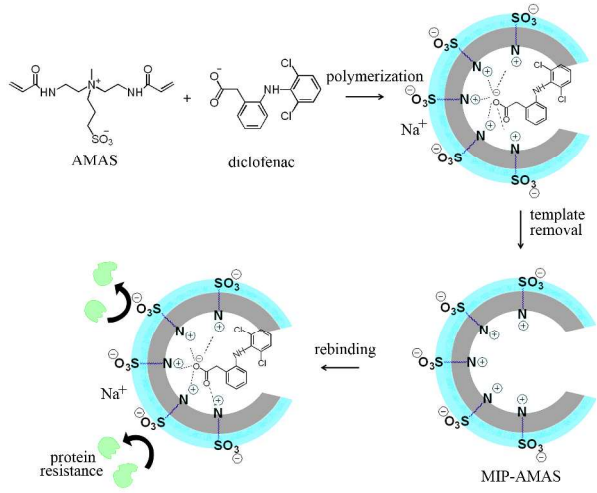


Fig. 1 (a) ^{1}H NMR spectrum of AMAS and (b) water-solubility of different crosslinkers (300 mg of each monomer dissolved in 1 mL water).

AMAS was synthesized via a facile two-step method as shown in scheme 1. First, amidation of 2,2'-diamio-N-methyldiethylamine was conducted with acryloyl chloride in dichloromethane at 0 °C. Then, 1,3-propane sultone was added and the final product was obtained through a ring-opening reaction. ^{1}H NMR (300 MHz, D_2O) spectrum of AMAS was shown in Fig.1(a): 6.16 (2 H, m), 5.71 (1 H, m), 4.70 (3 H, s), 3.59 (5 H, m), 3.12 (1 H, s), 2.89 (1 H, dd, J 12.8, 5.6), 2.16 (1 H, m), 1.97 (1 H, s). In addition, ^{13}C NMR, MS spectra of the AMAS, and ^{1}H, ^{13}C-NMR and MS spectra of the first step product were list in Fig.S1. The synthesis method of AMAS has simple procedure, fast reaction rate, mild reaction conditions, and high product yield (80%). Fig.1 (b) shows water solubility of the AMAS compared with three commonly used crosslinkers. Unlike ethylene glycol dimethacrylate (EDMA), poly (ethylene glycol) diacrylate (PEGDA, Mw 258), and N,N'-methylenebisacrylamide (MBA), which showed poor solubility in water, the AMAS possessed extremely high solubility in water. 300 mg of AMAS was completely dissolved in 1 mL water at 20 °C. To the best of our knowledge, as-prepared AMAS showed the superior water solubility compared to the known crosslinkers by far. The highly solubility of AMAS is attributed to the dissociation of zwitterionic groups. Thus, instead of using aprotic organic solvents such as toluene, acetonitrile and chloroform as porogen, MIP was ready to be made in pure aqueous solution using the AMAS as crosslinker and functional monomer.



Scheme 2 Schematic representation of the process for preparation of DFC imprinted polymers

To validate this, diclofenac (DFC), an emerging drug pollutant in waste water, was employed as a model template. DFC imprinted polymers were prepared in water with the new monomer by precipitation polymerization as illustrated in Scheme 2. First, DFC and AMAS were dissolved in 40 mL distilled water. After the mixture was stirred for 30 min, polymerization was initiated at 60 °C for 3 h. The template was removed by methanol/acetic acid (9:1,v/v) for three times. During the preparation, the content of AMAS as well as methylenebisacrylamide (MBA) in the MIPs was optimized and the results were shown in Fig. S2. SEM images (Fig.S3) and specific surface areas and pore volume of the resultant MIPs and NIPs were list and discussed in ESI. Adsorption capacity, selectivity, and resistance to nonspecific protein adsorption of the resulting MIPs were evaluated in aqueous medium.

To demonstrate the super water compatibility of the AMAS based MIPs (MIP-AMAS), 2-vinylpyridine (2-VP), a commonly used component for synthesis of DFC imprinted polymers in organic solvent, was employed for preparation of MIPs in water by the same procedure for comparison. Fig. 2a shows the amount of DFC bound to the MIPs and non-imprinted polymers (NIPs) at various initial concentrations. The adsorption capacities (Q, calculation method was shown in ESI) of the MIPs (MIP-AMAS, MIP-VP) were much higher than that of corresponding NIPs (NIP-AMAS, NIP-VP) for each of the different individual concentration values of DFC. Clearly, the MIP-AMAS revealed considerably higher maximum binding capacity (207 mg/g) for DFC in water than MIP-VP (68 mg/g). Scatchard analysis indicated that a low dissociation constant 2.16 mg/L for MIP was obtained (ESI, section 6.0). One of the reasons is that the AMAS based MIPs were more water compatible due to the hydrophilic zwitterionic monomer. Another important reason was that the newly synthesized monomer AMAS could act as a crosslinker as well as functional monomer simultaneously. Therefore, the resulting MIPs owned more imprinting sites at the equal cross-linking degree (1.20 mmol crosslinker). Furthermore, the kinetic uptake of DFC by the MIPs and NIPs was investigated, and experimental results showed that the adsorption equilibrium was reached after 10 min for both MIP and NIP (Fig.S4), which was much faster than that of typical imprinted materials. Therefore, it is very efficient for this material to be applied in practice.

Selectivity is an important factor to evaluate the imprinting efficiency of MIPs. In this study, mefenamic acid (MAC), ibuprofen (IBP) and sodium benzoate (SB) were employed as the competitive analogues of DFC. Molecular structures of the four analytes were list in Fig.S5 and the concentration of the analytes were measured by HPLC. As shown in Fig. 2b. The amount of template DFC adsorbed by the MIP-AMAS (197.4 mg g^{-1}) was found to be significantly higher than that of MAC (46.0 mg g^{-1}), IBP (19.5 mg g^{-1}) and SB (8.7 mg g^{-1}). While NIPs showed unbiased enrichment towards DFC, MAC, IBP and SB. The imprinting factor (IF) calculated by the equation $IF=Q_{MIP}/Q_{NIP}$ was used to evaluate the special selectivity of the MIPs. For the template DFC the IF was 6.9, which was much higher than the IF of MAC (4.4), IBP (1.9), and SB (1.4). The IF of MAC was higher than other two compounds, indicating that the MIP could recognize its structural analogues of DFC over other compounds tested. This is because the structure and functional groups of MAC was very close to the template. These results indicated that the MIPs possess a high level of selectivity for the template molecule DFC. The selectivity was promoted not only by the binding sites created by the template molecule effectively leaving a memory of its size and shape, but also by the strong electrostatic interactions that occurred between the target molecules and the AMAS. In contrast, only

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chemical interaction retained on the NIPs, and the selectivity was therefore comparable for DFC, MAC, IBP, and SB.

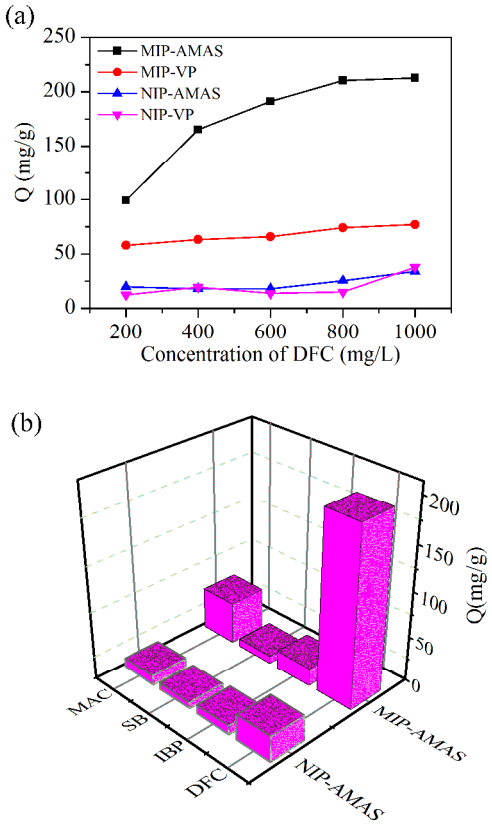

Fig. 2 (a) Static adsorption curves of the MIPs and NIPs for DFC. (b) Binding selectivity of the MIP-AMAS and NIP-AMAS.

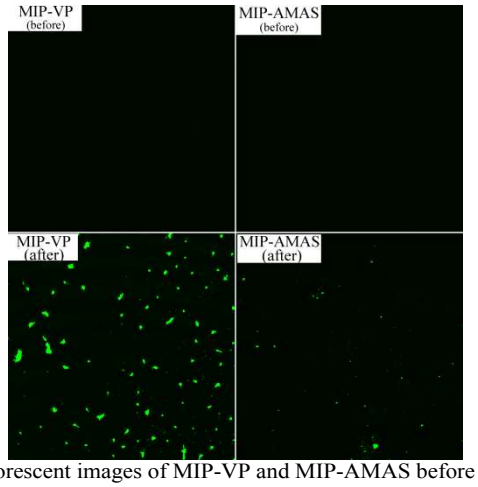

Fig. 3 Fluorescent images of MIP-VP and MIP-AMAS before (top) and after (bottom) rebinding with FITC-BSA.

Bovine Serum Albumin (BSA) was employed as a model protein interferent to examine the high protein resistance of MIP-AMAS. To visually reveal the function of AMAS resisting protein adsorption, BSA was labelled by fluorescein isothiocyanate (FITC). After the FITC-BSA was adsorbed by MIP-AMAS or MIP-VP, the fluorescent residue on MIPs was observed under fluorescence microscope (Olympus FV-1000) and the concentration of the BSA was measured by HPLC. As shown in Fig. 3, the intensity of fluorescence on MIP-VP was much stronger after incubating with FITC-BSA. The adsorption amounts of FITC-BSA reached 58.2 mg/g. However, after the MIP was modified with AMAS (MIP-AMAS), the fluorescence residue was almost invisible and the corresponding FITC-BSA adsorption amount was significantly decreased to 3 mg/g. To further demonstrate the low non-specific protein binding property of MIP-AMAS, a recovery study was carried out on the BSA samples (10 mg/mL) spiked with 10-60 mg L^{-1} of DFC. Experiment results (Table S1) showed that good recoveries from 92-95% were obtained. Generally, the presence of proteins always interfere the determination of small target compounds and the recovery would be very low. The high recovery in this work should attribute to the protein-resistant ability of AMAS. These results strongly indicate that the zwitterion monomer AMAS embedded in the MIPs showed high specificity and strong resistance to protein adsorption.

In summary, we have synthesized a triple-function (crosslinker, functional monomer and protein resistant monomer) zwitterion AMAS for preparing water compatible DFC imprinted polymer. The MIP-AMAS exhibited high imprinting efficiency for template in water due to the hydrophilicity and multi-functionalities of AMAS. Moreover, the resulting MIP-AMAS demonstrated strong resistance to non-specific protein adsorption in water phase owing to the zwitterionic groups of AMAS.

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Notes and references

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† Electronic Supplementary Information (ESI) available: detailed experimental procedures and analysis, NMR data, SEM data et al. See DOI:10.1039/b000000x/

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