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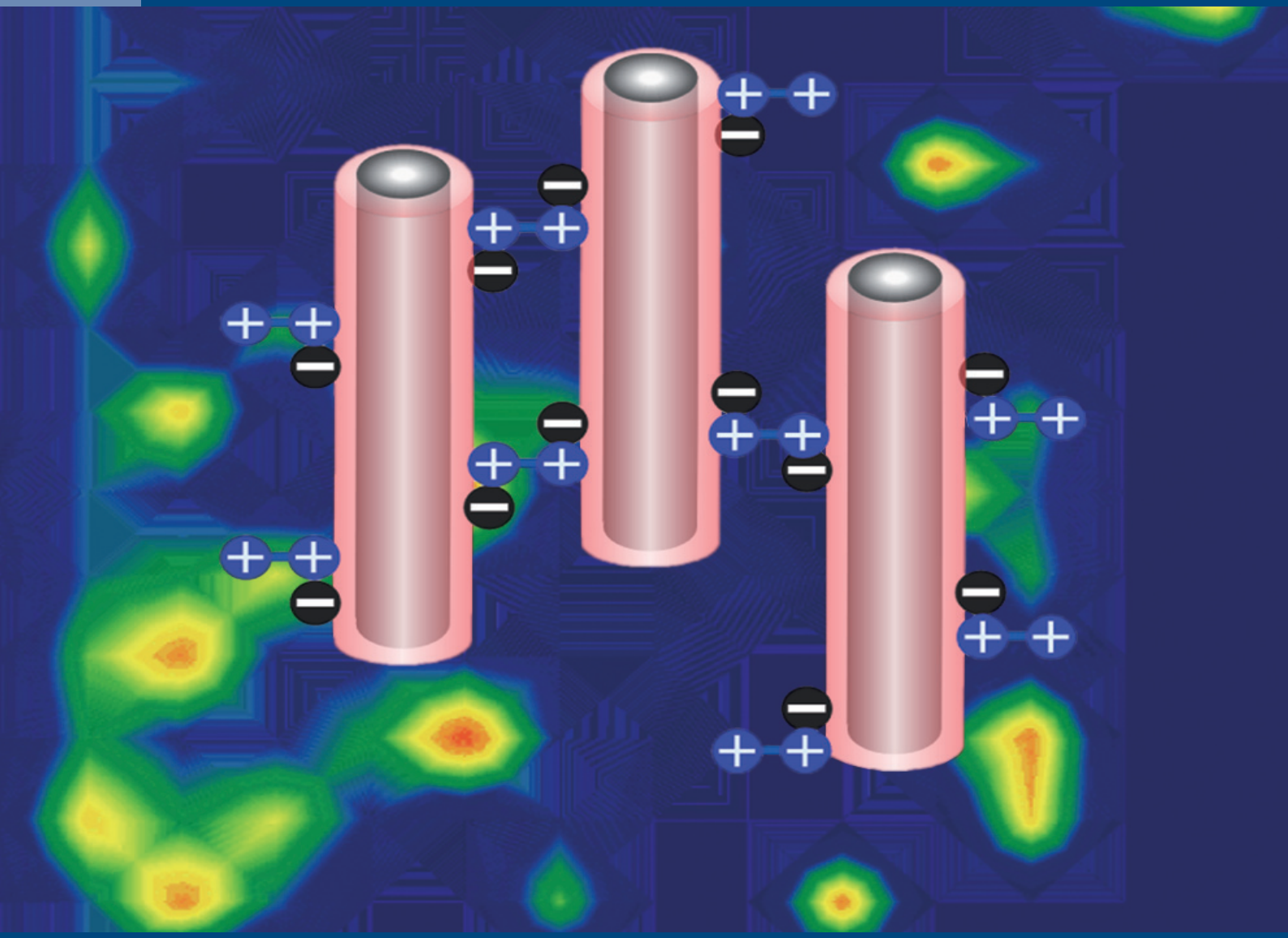

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RESEARCH ARTICLE

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Preparation of a stoichiometric molecularly imprinted polymer for auramine O and application in solid-phase extraction

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We describe a stoichiometric approach to the synthesis of molecularly imprinted polymers specific for auramine O. Using the stoichiometric interaction in molecular imprinting, no excess of binding sites is necessary and binding sites are only located inside the imprinted cavities. The free base of the template was obtained to facilitate the interaction with the monomers. Itaconic acid was selected as the functional monomer, and stoichiometric ratio of the interaction with the free base was investigated. The molecularly imprinted polymer preparation conditions such as cross-linker, molar ratio, porogen were optimized as divinylbenzene, 1:2:20 and chloroform/N,N-dimethylformamide, respectively. Under the optimum conditions, a good imprinting effect and very high selectivity were achieved. A solid-phase extraction method was developed using the molecularly imprinted polymers as a sorbent and extraction procedure was optimized. The solid-phase extraction method showed a high extraction recovery for auramine O in its hydrochloride form and free form compared to its analogues. The results strongly indicated that stoichiometric imprinting is an efficient method for development of high selectivity molecularly imprinted polymers for auramine O.

KEYWORDS
auramine O hydrochloride, molecularly imprinted polymers, rational design, solid-phase extraction, stoichiometric interactions

1 | INTRODUCTION

Auramine O hydrochloride (AOHCl) is a basic chemical dye, mainly used in dyeing of leather, cotton, paper, straw weave [1]. Toxicology data [2] shows that AOHCl has mild irritation effect on the skin mucous membrane, and can cause conjunctivitis, upper respiratory tract irritation, and dermatitis. People exposed to or inhaled with the dye are susceptible to be affected by its toxicity, and long-term intake of it leads to cancer. The international agency for research on cancer even referred the dye as a human carcinogenic compound [3,4]. It is of great importance to develop a selective sample pretreatment method for sensitive and accurate determination of AO in complex samples.

The main methods for the determination of AO include HPLC equipped with UV detector [5–7] and tandem mass spectrometry [8,9], TLC [10,11]. Other methods, such as real time mass spectrometry (RT-MS) [12] and Raman spectroscopy [1,13] were also reported. Except for TLC, other techniques require complex sample preparation and professional operation. Therefore, it is of great significance to establish a high selective sample pretreatment method for complex AOHCl samples.

Article Related Abbreviations: AO, auramine O; AOHCl, auramine O hydrochloride; DMF, N,N-dimethylformamide; DVB, divinylbenzene; EDMA, ethylene glycol dimethacrylate; IA, itaconic acid; MAA, methacrylic acid; MIP, molecularly imprinted polymer; NIP, non-molecularly imprinted polymer; TRIM, trimethylolpropane trimethacrylate

SPE [14] has been the most frequently used sample pretreatment technique in the last three decades due to its advantages of high enrichment factor, fast extraction rate, simple instrumentation and operation, low solvent consumption and easy combination with modern analytical instruments. SPE is faster and less labour intensive than traditional liquid-liquid extraction [15].

SPE is one of the most commonly used pretreatment methods for complex samples [16]. The type and properties of solid phase extraction sorbent directly affect the extraction efficiency. A large number of SPE sorbents are commercially available, such as C18, ion-exchange and size-exclusion resins, but they have the major disadvantages [14] of low selectivity, leading to co-extraction of interference components with the target analytes. With the advantages including low cost, easy preparation, high stability at extreme pH, temperatures, and reusability, molecularly imprinted polymers (MIPs) have attracted increasing attentions [17–19]. The application of SPE procedures applying MIPs was reported by Sellergren's group [20] for the first time. They made use of AIDS virus inhibitor pentamidine as a template to prepare MIPs for SPE and achieved good results. Since then, MI-SPE has been widely applied to environmental [21–23], clinical [24–26] and food [16,27–32] samples.

MIPs has the ability to selectively adsorb the template molecule from complex samples in presence of other molecules through interaction. Template bleeding is one of the biggest challenges for application of MI-SPE [33]. To avoid the risk of template bleeding, Andersson et al. [34] used a close structural analogue dummy template to substitute the real target molecule in 1997. Dummy template can better combine with a functional monomer and reduce bleeding of the template molecule. Chen et al. [35] prepared MIPs with trinitrophenol as a dummy template molecule for the detection of 2,4,6-trinitrotoluene (TNT). Elif et al. [36] used diethyl phthalate as a dummy template to synthesize dummy template molecularly imprinted microbeads as a SPE material to determine six phthalate esters in water by GC–MS. In our previous work [37], zidovudine ester was used as a dummy template to synthesize zidovudine MIPs.

The interaction between the template and functional monomer should be as strong as possible during the polymerization [38]. Experimental method [39,40] and computational modeling [41,42] are used in order to evaluate the interaction between template and functional monomer. The imprinting procedure is generally based on the linkage of suitable monomers containing functional groups to template molecules by some kind of interactions. When covalent interactions are used, monomers can be employed in the exact stoichiometric ratio to the template [43]. In non-covalent interactions, comparable strong association between the template and monomer can also be achieved at stoichiometric ratios [44]. In the result, a large number of solution phase complexes are produced and ultimately leads to the formation of imprinted binding sites after polymerization. Whitcombe et al. [45] determined monomer/template interactions by NMR titration and used its imprinted polymers to adsorb ampicillin from aqueous solutions. Holdsworth et al. [46] used a "stoichiometric" pyridine-based functional monomer to prepare MIPs using precipitation polymerization.

In this work, one kind of dummy template was prepared to synthesize imprinted polymers of AOHCl. The interaction between template and functional monomer was evaluated by the use of UV spectroscopic method. A stoichiometric interaction between the template and monomer was confirmed, and its ratio was obtained. Consequently, stoichiometric molecularly imprinted polymers against auramine O were successfully designed and synthesized for the first time. An SPE procedure was developed for selective extraction of AOHCl, using the MIPs of high affinity and specificity. The main idea of the work was described using a schematic form (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Materials

Reagents: Auramine O hydrochloride (AOHCl, 90%), 2,2-Azobisisobutyronitrile (AIBN), divinylbenzene (DVB), ethylene glycol dimethacrylate (EDMA) and methacrylic acid (MAA) were purchased from J&K Chemicals (Beijing, China). Itaconic acid (IA) was supplied by Sigma-Aldrich (Shanghai, China). Trimethylolpropane trimethacrylate (TRIM) was purchased from Acros Organics, USA. Ethanol, acetonitrile, glacial acetic acid, methanol, dimethylsulfoxide (DMSO) and N,N-dimethylformamide (DMF), 2,2-Azobisisobutyronitrile were obtained from Sinopharm Chemical Reagent. All the chemicals are analytical reagent except for mentioned.

Instrumentation: All reagents were weighed using an electronic analytical balance (BS210S, Beijing Sartorius Balance). Evaporation was achieved by rotary evaporator (RE-2000B, Shanghai Yarong Biochemistry Instrument Factory). The vacuum conditions were achieved by a vacuum oven (DZF-6020, Shanghai Jinghong Laboratory Instrument). Centrifugation was achieved with a high-speed centrifuge (HC-2062, Anhui USTC Zonkia Scientific Instruments). Constant temperature was achieved with electric thermostatic water bath (8002, Beijing Titan Instrument). UV-Vis spectroscopy was performed with the aid of UV-1800 spectrophotometer (Shimadzu, Japan). FTIR spectroscopy was performed using KBr pellets on a EQUINOX 55 (Bruker Optics, Germany) in the spectra range of 400–4000 cm^{-1}.

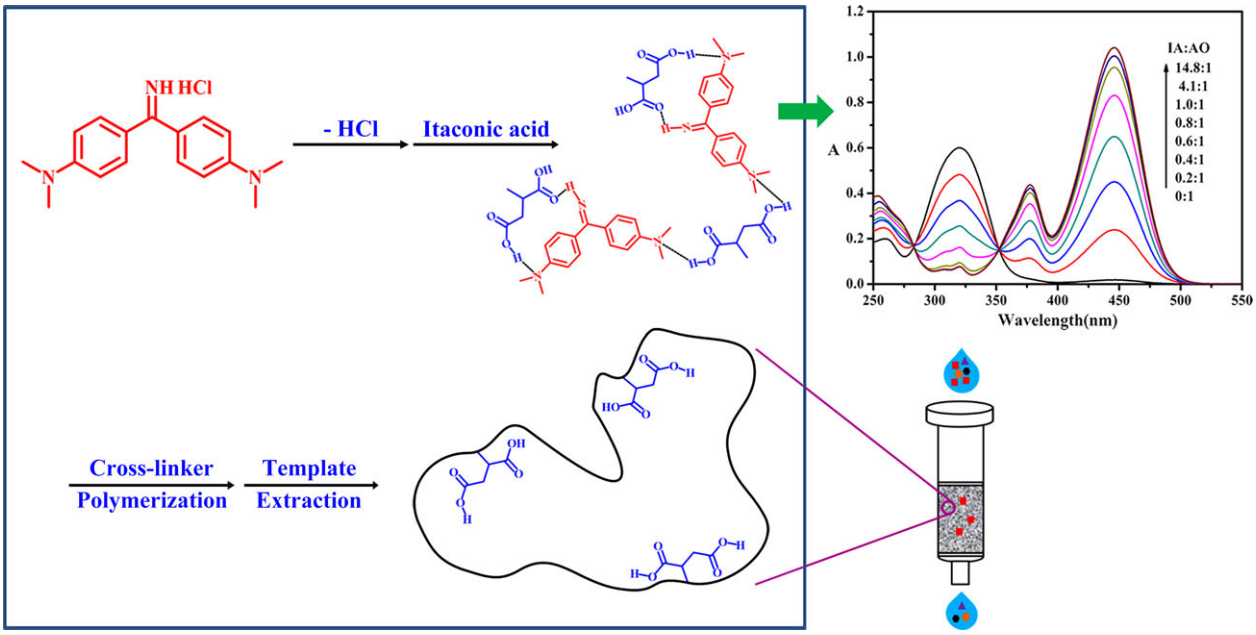

FIGURE 1 Synthesis scheme of stoichiometric MIPs for auramine O

2.2 | The evaluation of the template

Auramine O hydrochloride (AOHCl) is an acidic compound. In order to strengthen the binding ability of the template molecule and functional monomer, template molecules need to be neutralized, i.e., dummy template was prepared. Firstly, 1.0 g of AOHCl was dissolved in 100 mL of water. Then, the solution was sonicated for 15 min and the insoluble substances were removed by filtration. 0.5 mol/L Na_2CO_3 water solution was added to the filtrate to get a yellow precipitate. Yellow precipitate was collected by filtration. The yellow precipitate was dissolved in chloroform and then washed with water and dried with $MgSO_4$. Then the mixture was evaporated under reduced pressure. The dummy template AO was obtained as powder. The target molecule is often used in hydrochloride form to increase its water solubility. And the hydrochloride form is much more polar than the free form and not freely soluble in the organic solutions such as acetonitrile, chloroform and toluene, which are often used as porogen in MIPs.

2.3 | Spectroscopic evaluation of monomer template interactions

The proper selection of functional monomer is of great importance in molecular imprinting [14], because the interaction with functional groups affect the affinity of MIPs. In our previous work [39], the best functional monomer was chosen according to UV spectroscopic analysis. Currently, 0.748 mmol/L AOHCl acetonitrile solution, 0.658 mmol/L AO acetonitrile solution, 29.92 mmol/L IA acetonitrile solution and 29.92 mmol/L MAA acetonitrile solution were prepared, respectively. Then different proportions of template monomer mixed solutions were prepared. Then the absorbance at 442 nm was measured with a UV-Vis spectrophotometer to determine the optimum monomer and template.

Different solutions of template monomer mixtures were prepared, by mixing 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5 mL of AO acetonitrile solution (3.74×10^{-2} mmol/L) with 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 mL of acetonitrile solutions (3.74×10^{-2} mmol/L) of IA or MAA, respectively. Then, the absorbance of solution mixtures at 442 nm were measured with a UV-Vis spectrophotometer and the absorbances of the complex compound were obtained using the following equation:

$$A_C = A_F - \left(A_T + A_M\right) \quad (1)$$

where A_C, A_T and A_M represent the absorbance of complex, template and monomer, respectively. And A_F represents the absorbance of the mixture measured by UV-Vis spectrophotometer.

2.4 | Synthesis of molecularly imprinted polymers

According to Table 1, the templates and monomers were dissolved in a 30 mL glass tube, into which cross-linker and initiator were added. After complete dissolution, the mixture solution was degassed for 20 min in an ultrasonic bath and purged with nitrogen for 5 min to get rid of oxygen and sealed immediately. Then the polymerization solution was heated in an oil bath at 60°C under vacuum for 24 h. Each polymer was ground with a mortar and wet sieved with

TABLE 1 Composition of the polymerization mixtures used for preparation of the MIPs and NIPs for AOHCl

	n (template)/mmol		n(monomer)/mmol		n(cross-linker)/mmol					
Polymer	AOHCl	AO	MAA	IA	DVB	EDMA	TRIM	m(DMSO)/g	m(AIBN)/g	IF
1	1		4		20			3.252	0.0325	0.40
2	1			4	20			3.433	0.0343	1.10
3		1		1.5	20			3.102	0.0310	1.29
4		1		1.5		20		4.463	0.0446	0.72
5		1		1.5			20	7.261	0.0260	0.84
6		1		2	20			3.102	0.0310	1.41
7		1		2	20			3.174*	0.0626	1.83

*indicate that chloroform/DMF (3:1 w/w) mixture solution was used as solvent.

methanol to obtain polymer particles in the range of 38 to 75 μm. The resulting polymer particles were extracted with methanol/acetic acid (9:1 v/v) using a Soxhlet extractor until no template was detected. Finally, polymer particles were washed with methanol three times to remove acetic acid and dried at 60°C under vacuum for 24 h. For comparison, non-imprinted polymer was also prepared in the same way in the absence of template.

2.5 | Breakthrough experiment

2.5.1 | Preparation of SPE column

MIPs (100 mg) and NIPs (100 mg) were packed between polyethylene frits in 1 mL polypropylene SPE columns.

2.5.2 | Breakthrough curve

Breakthrough experiments were carried out following the procedure reported in our previous work [39] to evaluate binding capacity of the polymers. Firstly, SPE column was activated with 3 mL acetonitrile. Then 1 mL of 100 mg/L AOHCl acetonitrile solution was passed through the SPE column at a flow rate of 1 mL/min. Each filtrate was collected and its absorbance at 436 nm was measured. This process was repeated until the absorbance approached that of loading solutions. Breakthrough curves were then obtained and the binding capacity extrapolated from the 50% binding value. The maximum binding capacity of each polymer and the content of the analytes were calculated by the maximum adsorption volume and concentration.

2.6 | Extraction procedure

Molecularly imprinted SPE (MI-SPE) or non-molecularly imprinted SPE (NI-SPE) column was connected to a pump and activated sequentially with 1 mL of methanol and 1 mL of 30% acetonitrile water solution. And 1 mL of 100 mg/L AOHCl (or AO, chrysoidin) acetonitrile: water (3:7 v/v) solution was loaded onto the MI-SPE column at a flow rate of 1 mL/min. After washing with methanol: acetic acid (99.8:0.02 v/v) at the same flow rate, the analytes remained on the MI-SPE columns were eluted by methanol: acetic acid (9:1 v/v) to collect filtrate to determine the concentration of analyte.

2.7 | Batch adsorption

Six aliquots of 20 mg of MIPs and NIPs were weighed accurately and put in twelve 10 mL flasks respectively. And 5 mL of 10~200 mg/L AOHCl in acetonitrile: water (3:7 v/v) solution were added to each flask respectively. Then all flasks were placed in a shaker for 12 h at room temperature. Then the solutions were centrifuged, filtered, and the absorbance of filtrate was measured at 436 nm using UV-Vis spectrophotometer. Finally, the concentration of AOHCl was calculated according to the absorbance and the equilibrium adsorption capacity (Q, mg/g) was calculated using the following equation:

$$Q = (C_o - C_1) \times \frac{V}{m} \quad (2)$$

where C_0 and C_1 represent the initial solution and final solution concentration (mg/L) of AOHCl, respectively, V is the solution volume (L), and m (g) is the weight of the polymer.

3 | RESULTS AND DISCUSSION

3.1 | Modification of template molecule

In order to enhance the interaction between the template molecule and the functional monomer, AOHCl was treated with base solution, and HC1 part in the molecular was neutralized to obtain dummy template (auramine O, AO). The dummy template was characterized by UV-Vis spectrometry and Fourier transform infrared spectrometry (FTIR) (Supporting Information Figure S1). It can be seen that after neutralization, N-H absorptions found in the fields of 3500-3300 cm^{-1} and 2100-1900 cm^{-1} weaker than original ones. The characteristic absorption peak of C=N bond at 1700-1600cm^{-1} also become weak. This is probably due to decreasing of the electrostatic force between N-H and HC1.

The absorption spectra of both AOHC1 and AO were scanned in acetonitrile by UV-Vis spectrophotometer. It was found that the maximum absorption wavelength of AOHC1 shifted from 442 to 315 nm after the treatment. This is because there are three N atoms in the AO molecule which has lone pair electron to form n-π* transitions. The blue shift in absorption spectra is probably due to the change in n-π* transition before and after neutralization treatment. After neutralization, the energy of the non-bonding orbital (π*) in the molecule was greatly reduced, resulting in an n-π* transition of the electron, and the maximum absorption wavelength shift to blue.

3.2 | Spectroscopic evaluation of monomer template interaction

Ultraviolet spectroscopic analysis provides a means for determining the extent of monomer template complexation and allows a semi-empirical estimation of the number of selective recognition sites in a polymer prepared with a given monomer. This method provides an efficient tool for rapid optimization of molecularly imprinted polymers preparation. The spectrum of template monomer mixture in acetonitrile was used to evaluate the interaction between the template and monomer.

The ability of the template molecule and functional monomer to form a stable complex prior to polymerization is the key to achieving MIPs with high selectivity and high affinity. According to the experimental method in section 2.3, IA and MAA were served as functional monomer, and the interaction between the monomer and template was investigated. The results are shown in Figure 2.

Spectroscopic evaluation of the interaction between template AO and monomers was conducted in acetonitrile. As shown in Figure 2A, when AOHCl interacted with IA, there was no any new absorption peak was observed. In contrast, when AO interacted with the monomers (Figure 2B and Figure 2C), the maximum absorption wavelength of AO was shifted for 127 nm from its original value 315 nm, indicating that AO can form a stable complex with these two functional monomers. However, the absorption value at 442 nm is larger in the case of IA than that of MAA. This may be due to two reasons. One, IA molecule contains two –COOH groups, which can form strong hydrogen bonds with = NH of AO molecules (shown in Figure 1). Another, the acidity of IA is stronger ($pK_{a1} = 3.85$, $pK_{a2} = 5.44$) than that of MAA ($pK_a = 4.66$), so IA can form a strong electrostatic interaction with AO. Interacting with IA, AOHCl absorbance at 442 nm increased with the increasing of IA concentration, there was no any obvious change in the absorption spectra. It can prove that interaction of AOHCl with IA could not form a stable complex. Therefore, AO was chosen as the template molecule and IA was chosen as the functional monomer.

Job's plot method provides a means for determination of template-monomer molar ratio. A series of solutions in acetonitrile containing AO and IA (or MAA) were prepared

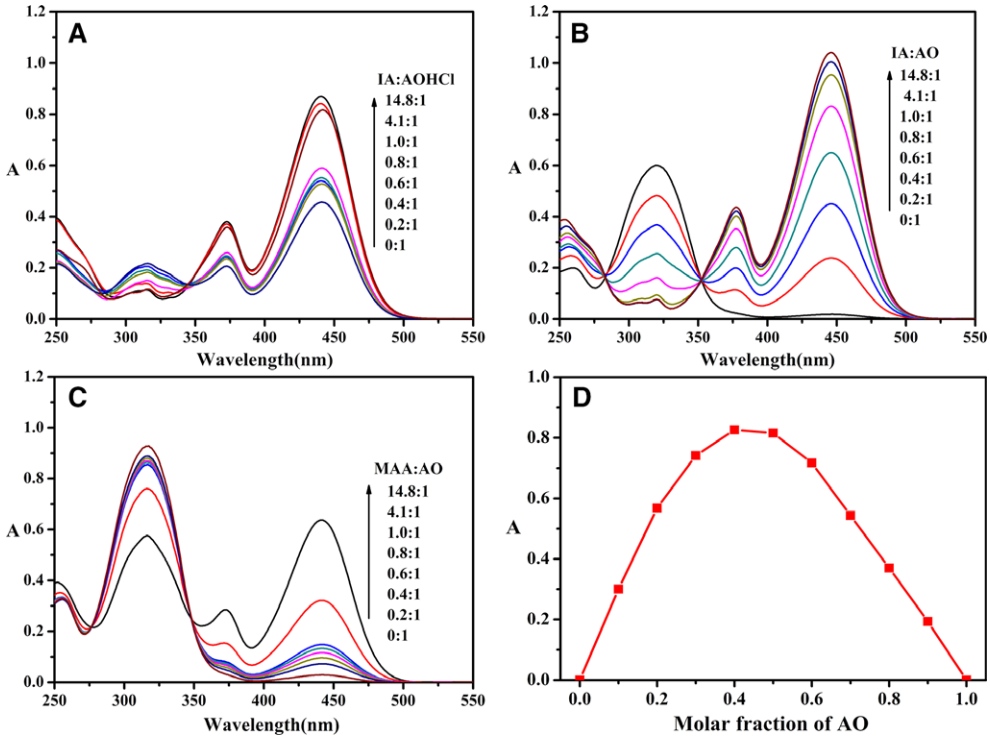

FIGURE 2 UV-Vis spectra of mixtures: (A) AOHCl and IA, (B)AO and IA, (C)AO and MAA (AOHCl: 0.748 mmol/L, AO: 0.658 mmol/L, MAA: 29.92 mmol/L, IA: 29.92 mmol/L) in acetonitrile at different monomer template mole ratio; (D) Job's Plot of the complexation between IA (3.74×10^{-2} mmol/L) and AO (3.74×10^{-2} mmol/L) in acetonitrile solution

and the total concentration remained constant (3.74×10^{-2} mmol/L). The Job's plot curve was plotted, as shown in Figure 2D. It can be seen that the absorbance of the AO-IA mixture increased gradually and reached to the highest at AO molar fraction of 0.4 and then decreased. The molar ratio of the template to monomer was found to be 1:1.5. However, there was no such a change observed in the interaction of AO with MAA, indicating that AO could not form a stable complex with MAA at this condition. Therefore, the optimal molar ratio of template AO to functional monomer IA was selected to be 1:1.5 and molecularly imprinted polymers were prepared using this ratio and farther investigated. In our new-published work [47], magnetic molecularly imprinted polymer of AOHCl was prepared with this ratio and used in extraction of AOHCl and its analogues from water.

3.3 | Selection of cross-linker

The selection of suitable cross-linker is of great importance in MIPs preparation. The type of cross-linker affects the position of the functional group on the polymer around the template molecule, thereby affects recognition ability of MIPs [48]. The specific recognition capacity of MIPs partly depends on the cross-linking degree of polymers. The cross-linker which has the smallest binding capacity believed to be the best cross-linker for a particular template, and most likely not to bring about non-specific binding. Cross-linkers are generally selected by experimental methods and computer simulations [37,42,49]. In this work, experimental method was chosen to select cross-linker. The homopolymers of DVB, TRIM, and EDMA were synthesized for selection of the most appropriate cross-linker. Homopolymers were examined by breakthrough test using 100 mg/L AOHCl acetonitrile solution. The binding percentage of homopolymer to AOHCl was calculated and the breakthrough curve was plotted as shown in Figure 3A and Supporting Information Figure S2. It can be seen from the breakthrough curve that DVB homopolymer has the least retaining amount of AOHCl at the first load, indicating that DVB homopolymer has the poorest binding to AOHCl. And the smallest binding to the template molecule was detected with DVB. It can be seen from Table 1 that DVB based polymers have relatively high imprinting factor. That is probably due to the lowest polarity among three cross-linkers, DVB gives the weakest interaction with the template, and could not interfere complex formation between the template and monomer. But the other two cross-linkers could compete with the functional monomer during the polymerization process, because of high binding capacity towards the template, so DVB was chosen as the most appropriate cross-linker.

3.4 | Molar ratio of cross-linker to monomer

Under the fixed ratio (1:1.5) of template to monomer, imprinted and non-imprinted polymers were synthesized using different ratio of functional monomer to cross-linker. SPE columns were prepared with these polymers and investigated with 100 mg/L AOHCl solution. Then the apparent adsorption capacities were measured from breakthrough experiment. Subsequently corresponding imprinting factors were obtained as shown in Figure 3C.

It can be seen from Figure 3 that the highest imprinting factor was achieved with cross-linker monomer ratio of 13.3. Therefore, this ratio was used for the preparation of imprinted polymers using DVB as well as EDMA and TRIM (polymer 3, 4 and 5). The polymers were found to have no imprinting effects, when EDMA and TRIM were used as cross-linker (Table 1). It is further confirmed that DVB is the most suitable cross-linker for the preparation of AOHCl imprinted polymers.

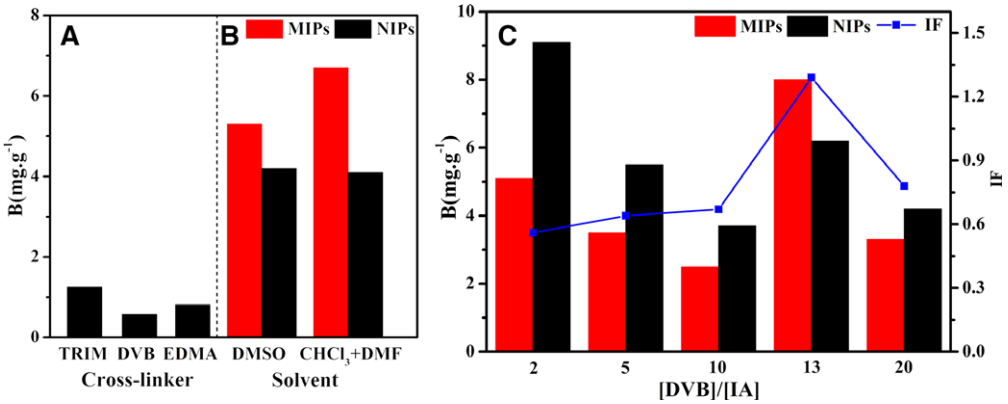

FIGURE 3 Binding capacity of AOHCl on the polymers (A) homopolymer of TRIM, DVB, and EDMA, (B) imprinted and non-imprinted polymers prepared in DMSO and chloroform/DMF respectively, (C) The effect of template cross-linker ratio on binding capacity and imprinting factor of the polymers. AOHCl acetonitrile (100 mg/L) and 100 mg of corresponding polymers were used

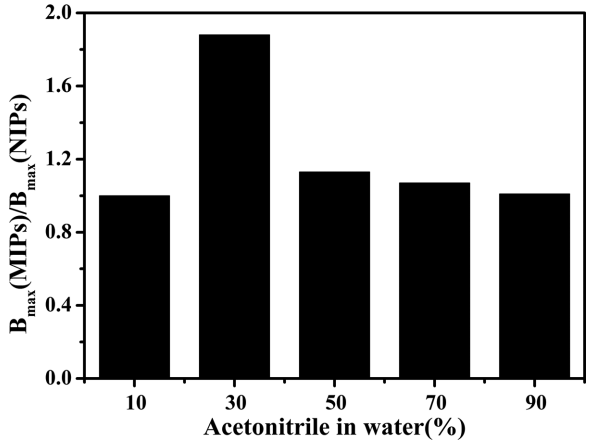

FIGURE 4 Effect of acetonitrile content in loading solvents on binding capacities

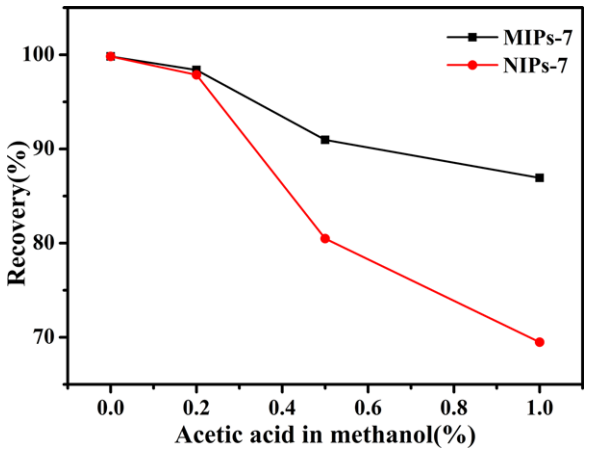

FIGURE 5 Effect of washing solvents on recoveries of AOHCl on MIPs and NIPs

3.5 | Selection of polymerization solvent

With the ratio of template/monomer/cross-linker of 1:2:20, DMSO and chloroform/DMF were used as polymerization solvents to prepare AOHCl imprinted polymers, respectively. After that corresponding SPE columns were prepared and 100 mg/L AOHCl acetonitrile solution was used in breakthrough experiment. The breakthrough curve and binding capacities of the polymers are shown in Supporting Information Figure S3 and Figure 3B respectively.

It can be seen from Supporting Information Figure S3 that MIPs-6 leaks more seriously than MIPs-7. It also can be seen in the Table 1 that imprinting factor of polymer 7 was higher than that of polymer 6. When chloroform/DMF (3:1 w/w) was used as polymerization solvent, polymer 7 showed better specificity to AOHCl. This is because the hydrogen bonding force between AO and IA was enhanced in chloroform. So, MIPs-7 was used for optimization of solid phase extraction procedure. The FTIR spectrum of MIPs-7 is shown in Supporting Information Figure S4.

3.6 | Optimization of SPE procedure

To improve the efficiency of SPE process, the loading conditions of MI-SPE procedure were optimized. Different ratios of acetonitrile and water were used as solvents to prepare AOHCl solution with a concentration of 100 mg/L. The MI-SPE column was loaded with AOHCl solutions to measure the breakthrough amount. The solvent ratios were plotted against ratio of adsorption capacity of MIPs and NIPs, as shown in Figure 4. The figure shows that when the acetonitrile content is 30%, there is the largest adsorption capacity ratio, so 30% acetonitrile/water was selected as the loading solvent in MI-SPE procedure.

Washing solvent was investigated to optimize MI-SPE procedure. Auramine O hydrochloride solution at a concentration of 10 mg/L was loaded and then washed with solvent mixtures of different ratios of methanol and acetic acid. The results are shown in Figure 5. It showed that with the increasing of the acetic acid content in solvent mixture, the retentions of AOHC1 on MIPs and NIPs decreased. When the amount of acetic acid is larger than 0.5%, the retention of AOHC1 by MIPs is less than 90%. To increase the recovery of AOHCl in practical applications, methanol containing 0.2% acetic acid was selected as the best washing media.

3.7 | Selectivity of polymers

Adsorption of AOHCl and its analogues (Supporting Inormation Figure S5) on the MIPs and NIPs were investigated using breakthrough experiment. As shown in Figure 6 and Supporting Information Figure S6, MIPs-7 exhibits a strong adsorption to the template molecule AO, followed by a good adsorption to AOHCl, and the worst adsorption to chrysoidin.

The apparent adsorption capacity of MIPs for AO, AOHC1, and chrysoidin were obtained from the breakthrough curve, and the relative separation factor α was calculated using the expression (3).

$$\alpha = \frac{Q_A}{Q_B} \tag{3}$$

where Q_A and Q_B represent the adsorption capacity of MIPs for the template or analogue molecules, respectively. The calculation results are shown in Table 2.

TABLE 2 The adsorption of MIPs for AO, AOHC1 and chrysoidin (n = 3)

Loading solution	Q(mg/g)	α	RSD(%)
AOHC1	10.00	1.0	4.1
AO	18.00	1.8	3.2
Chrysoidin	4.50	0.45	4.6

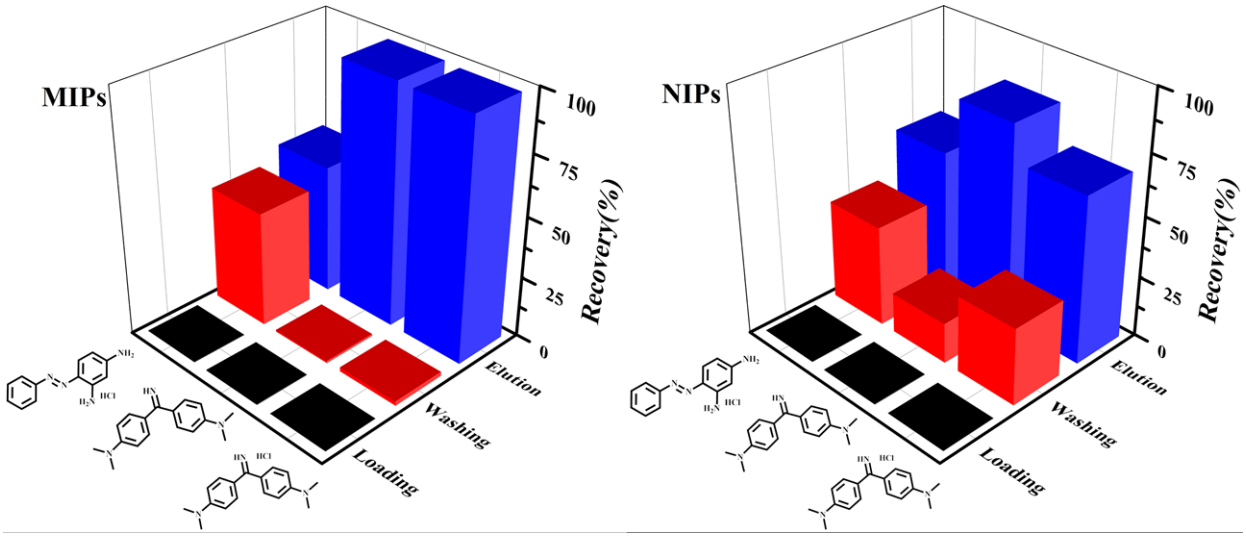

FIGURE 6 Extraction profiles for AOHCl, AO, Chrysoidin using MIPs and NIPs. (concentration of analytes: 100 mg/L, solvent: 30% acetonitrile water solution the experiment was done in triplicate)

It was observed that selectivity of MIPs towards AOHCl and AO were almost the same, and recoveries are 98.3% and 99.1% respectively. At the same time, much lower recovery (52.9%) was obtained for chrysoidin. It can be seen from the Figure 6 that chrysoidin was removed from MIPs and NIPs during the washing step. This is because the polymers have recognition sites that match AO structure, so that they have strong specific adsorption to AO. The structure of AOHC1 is very similar to AO, so MIPs have also selective adsorption to AOHC1. The result of selectivity test confirmed that the dummy template was effective in producing selective MIPs for AOHCl.

4 | CONCLUDING REMARKS

It was found that itaconic acid has strong interaction with the template molecule auramine O at the stoichiometric ratio of 1:1.5. The stoichiometric imprinted polymers using itaconic acid as functional monomer were synthesized. Conditions of MIPs preparation, such as cross-linkers, solvent were optimized, and DVB and acetonitrile were selected. The MIPs had good imprinting effect and high selectivity. An MI-SPE method was developed using the MIPs and extraction conditions were optimized. The method developed in this work was found to be useful for the preparation of selective sorbents for solid-phase extraction of auramine O.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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