Journal of Applied Polymer Science

Evaluation of Aminoquinoline-Imprinted Polymers and the Recognition Mechanism

Tieli Zhang,[1] Feng Liu,[2] Kean Li[2]

[1]Tangshan Key Laboratory of Biological and Chemical New Technology, Tangshan Teachers College, Tangshan, China
[2]The Key Laboratory of Bioorganic and Molecular Engineering, Ministry of Education, College of Chemistry & Molecular Engineering, Peking University, Beijing, China
Correspondence to: T. Zhang (E-mail: tielizhang@126.com)

ABSTRACT: Three molecularly imprinted polymers P(2-AQ), P(3-AQ), and P(8-AQ) based on methacrylic acid (MAA)–ethylene glycol dimethacrylate were prepared using isomers 2-aminoquinoline (2-AQ), 3-aminoquinoline (3-AQ), and 8-aminoquinoline (8-AQ) as template, respectively, by non-covalent bulk polymerization technique. Neither P(3-AQ) nor P(8-AQ) exhibited imprinting effect for 3-AQ or 8-AQ, whereas P(2-AQ) showed significant imprinting effect for 2-AQ. This indicates that the position of amino group on the quinoline ring has crucial influence on imprinting effect. The recognition mechanism of P(2-AQ) was investigated extensively by such methods as selective experiments, comparative study with 2-aminopyridine-imprinted polymer, and effect of different kinds of mobile phases. It is confirmed that there are complementary cavities in P(2-AQ) both in size and in the arrangement of functional groups to 2-AQ, and MAA binds 2-AQ via cyclic hydrogen bond. Furthermore, the influence of synthetic conditions on 2-AQ-imprinted polymers was explored. We found 2-AQ-imprinted polymer synthesized in acetonitrile porogen showed higher imprinting effect for 2-AQ than that prepared in chloroform. It is deduced that the morphology of the former might be more favorable to 2-AQ binding, which is also supported indirectly by the fluorimetric experiments estimating the interaction of 2-AQ with MAA in these two porogens. Additionally, 2-AQ-imprinted polymers prepared using two different amounts of acetonitrile exhibited very different imprinting effects, which suggested that porogen amount used in the imprinting process exerted significant influence. In addition to the in-depth elucidation of the recognition mechanism of 2-AQ-imprinted polymer, this article provides the basis for the separation and enrichment of bioactive 2-AQ. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 3447–3453, 2013

KEYWORDS: molecular recognition; properties and characterization; copolymers; cross-linking; radical polymerization

Received 14 November 2012; accepted 27 January 2013; published online 27 February 2013
DOI: 10.1002/app.39094

INTRODUCTION

Molecular imprinting is a widely accepted method for producing template specific polymers. It has been developing rapidly these years. Up to date, non-covalent approach is a predominantly used imprinting technique for the following reasons: since no covalent modification of the template molecule is required and a variety of different binding interactions can be used, non-covalent approach is both simple and general; the kinetics of the non-covalent binding is analogous to enzyme-substrate binding, and investigating the molecular recognition of molecularly imprinted polymers (MIPs) will be of significance for us to understand the biological molecular recognition processes. Furthermore, compared with biological antibodies, MIPs have unparalleled merits, such as ease of preparation and mechanical and chemical robustness. In addition to fundamental research interest,[1–6] MIPs have been found a wide range of applications in which high selectivity is desirable. There have been numerous reviews on these fields of applications, such as solid phase extraction, capillary electrochromatography, binding assays, and chemical sensors.[7–12]

This presentation is concentrated on two main objectives. One is to prepare imprinted polymer with high affinity and selectivity for 2-aminoquinoline (2-AQ), which is a bioactive compound originated from natural products.[13] The other is to take aminoquinolines and 2-aminopyridine (2-AP) as model compounds to explore the influence of such factors as template structure, porogen identity, and porogen amount on imprinting effect so as to get deep insight into the molecular recognition mechanism. Bearing these in mind, we adopted non-covalent bulk polymerization method to synthesize methacrylic acid–ethylene glycol dimethacrylate (MAA–EGDMA) based MIPs using 2-AQ, 3-aminoquinoline (3-AQ), 8-aminoquinoline (8-AQ),

Applied Polymer SCIENCE

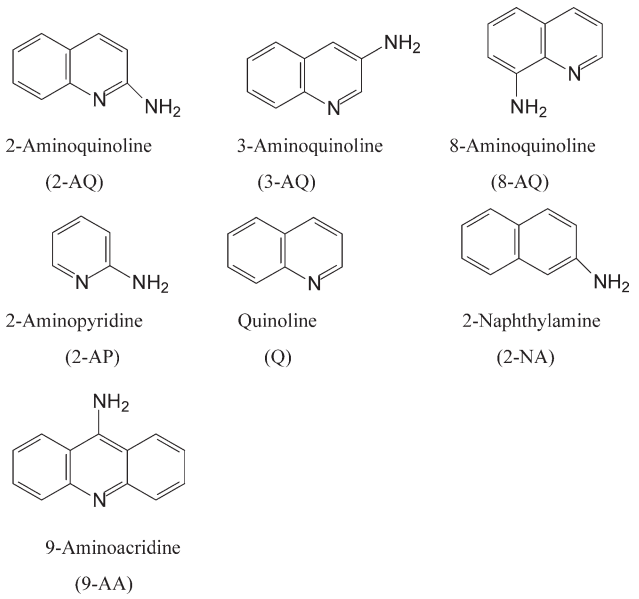

Figure 1. Chemical structures of the test compounds used in this study.

and 2-AP as the templates, respectively (Figure 1). These templates have in common planar-skeleton structures. 2-AQ, 3-AQ, and 8-AQ are different from each other only in the relative position of amino group on the quinoline ring. By investigating the molecular recognition properties of the three aminoquinoline isomer-imprinted polymers prepared under the same conditions, the influence of amino position on imprinting effect might be revealed. As we know, 2-AP is only one benzene ring less than 2-AQ. So, by comparative study on the molecular recognition properties of their corresponding imprinted polymers, we can not only discover the effect of template size on selective binding of the imprinted polymer but also find an alternative evidence for the molecular recognition mechanism of the 2-AQ-imprinted polymer to learn the size effect of template molecule. In addition, the imprinting effects of 2-AQ-imprinted polymers synthesized in two different porogens, acetonitrile and chloroform, were compared in order to explore the role of porogen used in the preparation of MIPs. Meanwhile, fluorimetric experiments estimating the interaction of 2-AQ with MAA in these two porogens were performed. Based on these experimental observations, the molecular recognition mechanism of the 2-AQ-imprinted polymer and the role of the porogens were elucidated. This work provides a new synthetic polymeric material with high affinity and selectivity for 2-AQ, which might be further used in the separation and enrichment of bioactive 2-AQ.[13]

EXPERIMENTAL

Materials and Reagents

2-AQ, 3-AQ, 8-AQ, 2-AP, quinoline (Q), 2-naphthylamine (2-NA), and chloroform were purchased from Beijing Chemical Plant (Beijing, China). 9-Aminoacridine (9-AA), EGDMA, methanol, and acetonitrile were from J&K Chemicals (Beijing, China). MAA was obtained from Yili Ltd. Co. of Refined Chemicals (Beijing, China). 2,2′-Azobisisobutyronitrile (AIBN) was from Shanghai Chemical Plant (Shanghai, China). Acetonitrile (MeCN) and methanol were of high performance liquid chromatography (HPLC) grade. All the other reagents were of analytical grade. EGDMA and MAA were distilled under vacuum to remove the inhibitor prior to polymerization. AIBN was purified by recrystallizing in ethanol.

Polymer Preparation

The components of the prepolymerization mixtures are shown in Table I. The reaction mixtures were sparged with nitrogen for 5 min and then sealed under vacuum. The polymerization was performed in a water bath at 60°C for 24 h. The resultant polymers were ground in a mortar and passed through a 30 μm sieve. Fine particles were removed by repeated decantation in acetone. The remaining particles were dried under vacuum at 60°C and used in the following studies. Each blank polymer (BP) was prepared in the absence of template and treated identically as for the corresponding imprinted polymer.

High Performance Liquid Chromatographic Evaluation

HPLC measurements were performed in a Hewlett-Packard 1100 series HPLC instrument (Palo Alto, California) equipped with a quaternary high-pressure pump and an UV–vis detector. A Rheodyne 7725i injection valve with a 20 μL sample loop was used. Polymer particles were packed into a standard stainless steel HPLC column (150 × 4.6 mm i.d.). The column was first washed with methanol–acetic acid (4 : 1, *v*/*v*) until a stable baseline was obtained to ensure the removal of the template compound. Then, it was washed with methanol to remove the

Table I. Components of the Prepolymerization Mixtures for the Preparation of Polymers

Polymer	Template	Amount of template (mmol)	Amount of MAA (mmol)	Porogen	Amount of porogen (mL)	Amount of EGDMA (mmol)	Amount of AIBN (mg)
P(2-AQ)	2-AQ	1	4	$CHCl_3$	15	30	60
P(3-AQ)	3-AQ	1	4	$CHCl_3$	15	30	60
P(8-AQ)	8-AQ	1	4	$CHCl_3$	15	30	60
P(2-AP)	2-AP	1	4	$CHCl_3$	15	30	60
BP	–	–	4	$CHCl_3$	15	30	60
P1	2-AQ	1	4	MeCN	15	30	60
BP1	–	–	4	MeCN	15	30	60
P2	2-AQ	1	4	MeCN	7.5	30	60
BP2	–	–	4	MeCN	7.5	30	60

Table II. Retention of Test Compounds on P(2-AQ), P(2-AP), and BP in Methanol

Parameter	2-AQ	2-AP	3-AQ	8-AQ	Q	2-NA	9-AA
$k'_{P(2\text{-}AQ)}$	27.3	10.2	0.86	0.97	0.59	1.16	5.72
k'_{BP}	5.35	2.72	0.65	0.96	0.41	1.16	4.25
$IF_{P(2\text{-}AQ)}$	5.10	3.74	1.32	1.01	1.44	1.00	1.35
$\alpha_{P(2\text{-}AQ)}$	1	2.69	31.8	28.2	46.3	23.5	4.77
α_{BP}	1	1.97	8.23	5.57	13.0	4.61	1.26
$f_{P(2\text{-}AQ)}$	1	1.37	3.86	5.05	3.55	5.10	3.79
$k'_{P(2\text{-}AP)}$	9.97	13.3	0.86	1.15	0.44	1.19	3.17
$IF_{P(2\text{-}AP)}$	1.86	4.90	1.32	1.20	1.07	1.03	0.75
$\alpha_{P(2\text{-}AP)}$	1.34	1	15.5	11.6	30.3	11.2	4.21
α_{BP}	0.51	1	4.18	2.83	6.63	2.34	0.64
$f_{P(2\text{-}AP)}$	2.63	1	3.71	4.09	4.57	4.78	6.58

residual acetic acid in the column. Subsequent chromatographic analysis was executed with the selected mobile phase at a flow rate of 1.2 mL min^{-1}. Each substrate was monitored at 260 nm absorption wavelength on the UV detector except 2-AQ monitored at 235 nm. The substrate volume and concentration injected was 20 μL and 1 m*M*, respectively. Each substrate was injected independently. The capacity factor, k', was calculated from the equation $k' = (t_R - t_0)/t_0$, where t_R is the retention time of a substrate and t_0 is the time to elute the void marker, acetone. The separation factor, α, was found from the equation $\alpha = k'_t/k'_i$, where k'_t is the capacity factor of the template, and k'_i is that of a test substrate. In this study, imprinting factor (IF) and imprinting selectivity factor (f) were adopted to evaluate the molecular imprinting effect.[4] They were calculated according to the following formulae, $IF = k'_{MIP}/k'_{BP}$ and $f = \alpha_{MIP}/\alpha_{BP}$, where k'_{MIP} and α_{MIP} are the capacity factor and the separation factor of a substrate on MIP, and k'_{BP} and α_{BP} represents the capacity factor and the separation factor on BP, respectively. IF and f reflects the affinity enhancement of MIP for the substrate and the selectivity enhancement for the template, respectively, because of template effect.

Fluorimetric Measurement

The concentration of 2-AQ was fixed at 0.1 m*M*. MAA–2-AQ mixture solution with the molar ratio of [MAA]/[2-AQ] = 4 was prepared. The fluorescence emission spectra for 2-AQ, MAA–2-AQ, and MAA in $CHCl_3$ or MeCN solutions were recorded at the excitation wavelength of 333 nm on a Hitachi-F-4500 fluorescence spectrometer with a high-pressure xenon lamp and a 1.0 cm quartz cell (Tokyo, Japan).

RESULTS AND DISCUSSION

Influence of Template Structure on Imprinting Effect

In order to test the influence of template structure on imprinting effect, three MIPs P(2-AQ), P(3-AQ), and P(8-AQ) were prepared under the same conditions using 2-AQ, 3-AQ, and 8-AQ as template, respectively (Table I). The structural difference of these three templates is only the position of the amino group on the quinoline ring. Chromatographic evaluation was carried out to test the ability for them to rebind their own templates. And the results showed that P(3-AQ) and P(8-AQ) had no imprinting effect because the retention values of 3-AQ and 8-AQ on P(3-AQ) and P(8-AQ) had no difference compared with those on BP. However, P(2-AQ) has high imprinting effect for 2-AQ (data shown in Table II). The following is our explanation. As we know, the molecular recognition ability of MIP is the cooperation of the following two processes, in both of which the template plays an important role. First, template and monomer should have strong interaction in the polymerization process in order to produce enough molecular recognition sites; second, the template should be able to rebind to the MIP in the recognition process. Therefore, the interaction between template and monomer determines the molecular recognition ability of MIP. The above three positional isomer templates 2-AQ, 3-AQ, and 8-AQ are basic compounds, and the corresponding p*Ka* is 7.34, 4.95, and 3.99, respectively. According to literature report,[14] the association strength between the substituted pyridine and carboxylic acid increases with the basicity of the former increasing. So, it is expected that the alkalinity of the three aminoquinoline isomers will affect their association strength with MAA. The strong interaction between aminoquinoline and MAA will be favorable for fabricating high specific sites in the MIP for the template-rebinding.[4,15] Thus, we can deduce that among the three aminoquinolines the most alkaline 2-AQ can associate with MAA most strongly to produce high specific sites for 2-AQ. In addition, according to literature reports,[16,17] when hetero-atom nitrogen and amino group connected to the ring are at ortho position, they can interact with MAA via strong cyclic hydrogen bonding to obtain MIPs with high affinity and selectivity. Therefore, the high binding and selectivity of P(2-AQ) for 2-AQ should be attributed to the strong cyclic hydrogen bonding between 2-AQ and MAA. Weaker interaction between 3-AQ or 8-AQ and MAA is predicted because the basicity of either 3-AQ or 8-AQ is weaker than that of 2-AQ. On the other hand, because both 3-AQ and 8-AQ are unable to form stable cyclic hydrogen bond with MAA, they interact with MAA through single hydrogen bond, which is also unfavorable for their strong association with MAA. For these two reasons, P(3-AQ) and P(8-AQ) had no molecular imprinting effect. All in all, the results indicate that the position of amino group on

Applied Polymer SCIENCE

the quinoline molecular skeleton has vital influence on the molecular imprinting effect, and the high imprinting effect of 2-AQ-imprinted polymer is attributed to the strong cyclic interaction between 2-AQ and MAA.

Molecular Imprinting and Recognition Mechanism of P(2-AQ)

Selective Experiments. Several structurally related analogs of 2-AQ (Figure 1) were chosen as substrates to test the selectivity of P(2-AQ), from which we could gain insight into the molecular recognition mechanism of P(2-AQ). The chromatographic results are summarized in Table II. It can be seen from Table II that P(2-AQ) exhibits the highest IF value for 2-AQ among the substrates, which indicates that P(2-AQ) has the highest specific binding to 2-AQ. The high f values for the other substrates on P(2-AQ) show that the selectivity of P(2-AQ) for 2-AQ is enhanced markedly because of its imprinting effect. Our explanation is as follows. There are complementary cavities to 2-AQ in P(2-AQ) and 2-AQ might bind the residual –COOH imprinted on P(2-AQ) via cyclic hydrogen bond.[16] Among the test compounds, 3-AQ, 8-AQ, Q, and 2-NA are almost of the same size as 2-AQ, but they bind the residual –COOH imprinted on P(2-AQ) via weak single hydrogen bond, and on the other hand, the single hydrogen bond will be weakened greatly in the mobile phase of methanol. Thus these substrates exhibit low IF values. In another word, P(2-AQ) has no apparent specific binding to them. As for 9-AA, which is much larger than 2-AQ, its IF value is low because its larger size makes it difficult to enter into the 2-AQ-imprinted cavities. Interestingly, 2-AP also exhibits high IF value on P(2-AQ), which indicates that P(2-AQ) can also recognize 2-AP. The following is our explanation. 2-AP is of the same planar-skeleton structure as 2-AQ and only one benzene ring less than 2-AQ. Thus, 2-AP can enter into 2-AQ-imprinted cavities and binds to the arranged –COOH groups via cyclic hydrogen bond because of its smaller size and the same arrangement of the functional groups as 2-AQ. So, P(2-AQ) shows high specific binding ability for 2-AP. In conclusion, selective experimental results confirm that there are cavities complementary to 2-AQ in both shape and functional group array in P(2-AQ).

Contrast Experiment of P(2-AQ) and P(2-AP). In this study, we introduced an original indirect alternative method to further confirm that there are cavities complementary to 2-AQ in P(2-AQ). As we have mentioned above, both 2-AP and 2-AQ have in common the 2-AP moiety in their molecular structures, and 2-AP is only one benzene ring less than 2-AQ. So, their hydrogen bonding interaction patterns with MAA should be the same, i.e., forming cooperative hydrogen bond. Investigation of the molecular recognition properties of 2-AP-imprinted polymer will be helpful for us to elucidate whether or not there are 2-AQ-imprinted cavities complementary to 2-AQ in P(2-AQ). Based on this consideration, we prepared P(2-AP) using 2-AP as the template molecule under the same conditions as P(2-AQ). The chromatographic results (Table II) show that P(2-AP) preferentially binds 2-AP. 2-AP has the highest k' and IF value on P(2-AP), indicating that P(2-AP) has imprinting effect for 2-AP. As we have discussed (Table II), P(2-AQ) showed the highest specificity for 2-AQ (IF = 5.10) and also relatively high specificity for 2-AP (IF = 3.74), suggesting that P(2-AQ) has a significant level of cross-reactivity for 2-AQ and 2-AP because of their very similar structures, especially having the same interacting pattern with MAA. However, P(2-AP) exhibited very different specificity for 2-AP and 2-AQ. Because of the imprinting effect of 2-AP, P(2-AP) showed the highest specificity for 2-AP (IF = 4.90) but much lower specificity for 2-AQ (IF = 1.86), indicating that the imprinted cavities in P(2-AP) were smaller than those in P(2-AQ). The smaller size of 2-AP than that of 2-AQ should be responsible for it. This fact proves that the size of the cavities created in the case of P(2-AP) is fit only for 2-AP molecule, and it is difficult for the larger molecule 2-AQ to enter into. The lower IF value of 2-AQ on P(2-AP) compared with that obtained on its own polymer P(2-AQ) is most likely due to the difference in the imprinted cavity size. The above results provide an indirect evidence that there are complementary cavities to 2-AQ in P(2-AQ), i.e., the specific sites are complementary in size and functional group arrangement to 2-AQ. In addition, the contrast experiment not only demonstrates that the high selective binding imprinted polymer could be prepared for 2-AP, but also gives a good example to reveal the effect of template size on imprinting selectivity of MIPs and to elucidate the imprinting mechanism. A smaller size compound could be retained and recognized by the larger one-imprinted polymer if the structure of the larger template compound is analogous to the smaller one, i.e., their interacting groups are the same.[18] This strategy might also provide a potential alternative route for the selection of mimic template compound in MIP solid phase extraction.

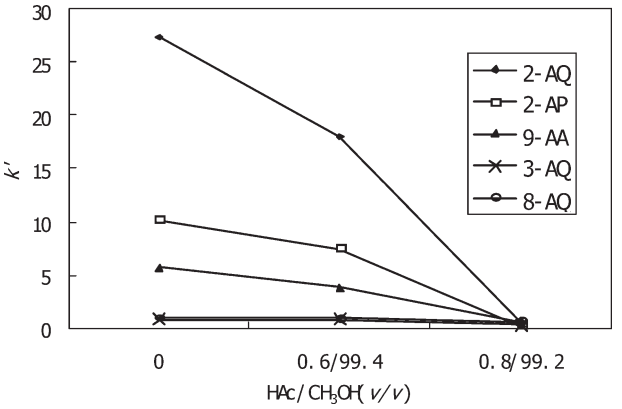

Figure 2. Effect of HAc content in methanol on the retentivity of P(2-AQ).

Influence of Mobile Phase on the Retentivity of P(2-AQ). To demonstrate the interaction in the recognition process of P(2-AQ), three mobile phases with different amounts of acetic acid–methanol solutions were employed to probe binding affinities of P(2-AQ) to some of the substrates (Figure 2). We can see from Figure 2 that the capacity factors of the substrates are strongly influenced by the contents of acetic acid. Both the binding affinity and the selectivity decreased with the acetic acid content increasing. As we know, acetic acid has strong hydrogen binding ability, and it will compete with the substrate for the binding sites, which leads the retention and selectivity of P(2-AQ) to decrease. This confirmed that hydrogen bonding played an

Table III. Capacity Factors (k') of Test Compounds on P(2-AQ) in the Mobile Phase of HAc/MeCN (3 : 97, *v*/*v*)

Parameter	2-AQ	2-AP	3-AQ	8-AQ	9-AA
$k'_{P(2\text{-}AQ)}$	18.1	6.30	3.01	0.97	0.60
k'_{BP}	1.58	0.63	0.88	0.79	0.36

important role in the specific binding of P(2-AQ). In order to further verify the above selective binding mechanism of P(2-AQ), we performed the binding experiments utilizing nonprotic solvent acetonitrile instead of the protic solvent methanol. We found that 2-AQ could not be eluted in the time span of 60 min when pure acetonitrile or HAc/MeCN = 1/99 (*v*/*v*) was used as the mobile phase. However, as we have known, only a small amount of acetic acid in methanol, e.g., HAc/CH_3OH = 1/99 (*v*/*v*) will make 2-AQ have no retention. This demonstrates that P(2-AQ) binds 2-AQ more strongly in acetonitrile than in methanol solution. It further supports that the main binding interaction of P(2-AQ) to 2-AQ is hydrogen bonding. The stronger ability of methanol than that of acetonitrile in competing with 2-AQ for the binding sites of P(2-AQ) can account for the weaker retention of 2-AQ in methanol than that in acetonitrile. Table III shows the capacity factors of some test compounds on P(2-AQ) in the mobile phase of HAc/MeCN = 3/97 (*v*/*v*).

Effect of Synthetic Condition on 2-AQ-Imprinted Polymer

Influence of Porogen Identity. It is well known that the porogen employed during polymerization will affect the interaction strength between monomer and template, and thus, the porogen will affect the capability of the imprinted polymer to rebind the template.[19] In this study, we chose acetonitrile and chloroform as two kinds of porogens with different polarities to investigate the influence of porogen identity on the molecular recognition ability of 2-AQ-imprinted polymer. P1 was prepared in the same way as P(2-AQ) except using acetonitrile instead of chloroform as porogen (Table I), and the molecular recognition performance of P1 was investigated in the same way as that of P(2-AQ). As P(2-AQ), P1 exhibits the highest affinity (k') and IF value for 2-AQ among the test compounds (Table IV) owing to the imprinting effect of P1 for 2-AQ. By comparison of k' (2-AQ), IF (2-AQ), and f values on P1 with the corresponding values on P(2-AQ), it is found that the values on P1 is larger than those on P(2-AQ). This indicates that P1 has higher imprinting effect for 2-AQ than P(2-AQ). But theoretically, the interaction of MAA and 2-AQ should be stronger in the weak-polar solvent chloroform than in the medium-polar solvent acetonitrile in the polymerization process. In consideration of these, we speculate that the morphology of P1 imprinted in acetonitrile might be more favorable to binding 2-AQ. According to the literature,[20] the morphologies of imprinted polymers prepared in acetonitrile and chloroform were different. As a matter of fact, we also found that the imprinted polymer prepared in chloroform was much harder than that synthesized in acetonitrile. This result demonstrates that two factors should be considered to choose an appropriate porogen in the preparation of MIP with high imprinting effect. First, functional monomer and template should have a strong interaction in the porogen; second, the different morphologies of MIPs prepared in different porogens should be taken into account, which might influence the imprinting effect.[19] So, we should take into a comprehensive consideration for the selection of an appropriate porogen.

Influence of Porogen Amount. The influence of porogen amount utilized in the polymerization process on the imprinting effect was investigated. P2 was prepared by adding 7.5 mL acetonitrile instead of 15 mL acetonitrile in the preparation of P1 (Table I). The chromatographic results are shown in Table IV. The results indicate that the amount of porogen exerts remarkable influence on the recognition performance of 2-AQ-imprinted polymer. Thus, it is concluded that the amount of porogen is an important factor to be optimized for the preparation of MIP with high affinity and selectivity. In fact, the effect of porogen amount reflects the influence of the concentrations of the constituents in the prepolymerization mixture on imprinting effect,[21–23] which might from equilibrium point of view affect the stability of the template-monomer complex and thus affect the number of specific binding sites. Also, we expect that P2 would be a very promising selective adsorbent for the

Table IV. Retention of Test Compounds on P1 and P2 in Methanol

Parameter	2-AQ	2-AP	3-AQ	8-AQ	Q	2-NA	9-AA
k'_{P1}	32.8	8.67	0.64	0.68	0.44	0.72	4.87
k'_{BP1}	3.66	1.69	0.30	0.51	0.20	0.59	2.90
IF_{P1}	8.96	5.13	2.13	1.33	2.20	1.22	1.68
α_{P1}	1	3.78	51.3	48.2	74.6	45.6	6.74
α_{BP1}	1	2.17	12.2	7.18	18.3	6.20	1.26
f_{P1}	1	1.75	4.20	6.72	4.07	7.34	5.34
k'_{P2}	64.8	13.00	0.89	1.78	0.53	0.97	8.33
k'_{BP2}	4.64	2.29	0.53	0.94	0.37	0.95	3.10
IF_{P2}	14.0	5.68	1.68	1.89	1.43	1.02	2.69
α_{P2}	1	4.99	72.8	36.4	122	66.8	7.78
α_{BP2}	1	2.03	8.75	4.94	12.5	4.88	1.50
f_{P2}	1	2.46	8.32	7.38	9.75	13.7	5.20

Applied Polymer SCIENCE

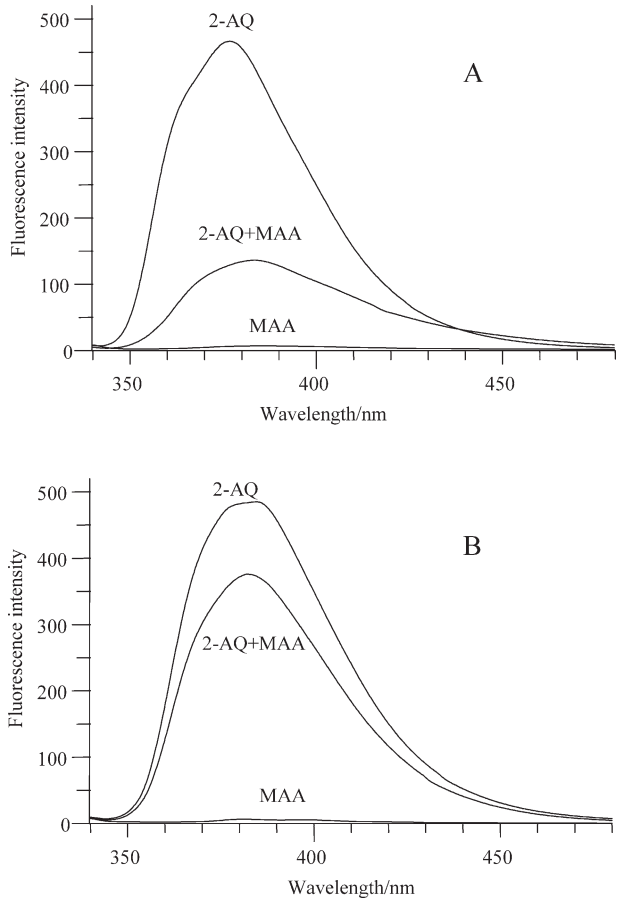

Figure 3. Fluorescence emission spectra of 2-AQ and MAA systems in chloroform (A) and acetonitrile (B) solutions. λ_{ex} = 333 nm; [2-AQ] = 0.1 m*M*; [MAA] = 0.4 m*M*.

separation and enrichment of 2-AQ and 2-AP because of its higher selective binding to both 2-AQ and 2-AP.

Investigation of the Interaction between 2-AQ and MAA in Solution

As we have known, the interaction between template molecule and monomer in the prepolymerization mixture is a crucial factor in a successful imprinting protocol. Fluorimetric method has been successfully used to study template/monomer interaction for the selection of an optimal functional monomer for a given template.[24] In the present study, we adopted fluorimetric method to probe the interaction between 2-AQ and MAA in the two different porogens of acetonitrile and chloroform (Figure 3). We can see from Figure 3 that the fluorescence intensity of 2-AQ has a notable decrease when MAA was added to 2-AQ in acetonitrile or chloroform solution. Thus, the presence of 2-AQ–MAA complex in both of the porogens was corroborated by fluorimetric experiments.[24] And we can also notice that the fluorescence lowering magnitude is larger in chloroform solution than that in acetonitrile solution, suggesting that 2-AQ binds MAA more strongly in chloroform than in acetonitrile in the prepolymerization solution.[24] This is consistent with the theoretical predication, i.e., the lower polarity of chloroform is favorable to the formation of more stable 2-AQ–MAA complex. Therefore, it seems difficult to explain why the polymer imprinted in acetonitrile exhibits better recognition for 2-AQ from the viewpoint of stability of 2-AQ–MAA complex. The above result further supports our inference that the morphologies of 2-AQ-imprinted polymers might be responsible for the molecular recognition performances.[25,26]

CONCLUSIONS

Three aminoquinoline isomer-imprinted polymers prepared utilizing MAA as monomer showed very different imprinting effects. Among them, only 2-AQ-imprinted polymer exhibited high imprinting effect to its template molecule 2-AQ. This indicates that the position of amino group in the aminoquinoline molecule has decisive influence on the imprinting effect, and the strong cyclic interaction between 2-AQ and MAA is favorable to the high imprinting effect of 2-AQ. Through various methods, the molecular imprinting and recognition mechanism of P(2-AQ) was elucidated. There are complementary cavities in P(2-AQ) both in size and in the arrangement of functional groups to 2-AQ, and MAA binds 2-AQ via cyclic hydrogen bonding. It must be emphasized that the comparative study of 2-AP- and 2-AQ-imprinted polymers not only provides the undoubted recognition mechanism for them but also reveals that a smaller size compound 2-AP could be retained and recognized by the larger size compound 2-AQ-imprinted polymer because of their similar structure, especially the same functioning groups. The study of the recognition mechanism of 2-AQ-imprinted polymer would be a good example to understand the imprinting and recognition phenomena of MIPs. The influences of different synthetic conditions on the imprinting effects of 2-AQ-imprinted polymers indicated that the nature and the amount of the porogen used in the imprinting process exerted great influence on the imprinting effect. In the selection of suitable porogen, we should consider its comprehensive effects both on the stability of template-monomer complex and on the morphology of the imprinted polymer. The 2-AQ-imprinted polymer synthesized in the porogen of acetonitrile exhibited high affinity and selectivity for 2-AQ, and this provides the foundation for the separation and enrichment of bioactive 2-AQ present in natural products.

ACKNOWLEDGMENTS

This work was financially supported by the Doctoral Scientific Research Fund of Tangshan Teachers College (No. 04D02) and the fund of Scientific and Technological Bureau of Tangshan City (No. 04360701B-6).

REFERENCES

1. Nicholls, I. A.; Andersson, H. S.; Charlton, C.; Henschel, H.; Karlsson, B. C. G.; Karlsson, J. G.; O'Mahony, J.; Rosengren, A. M.; Rosengren, K. J.; Wikman, S. *Biosens. Bioelectron.* **2009**, *25*, 543.
2. Simon, R.; Collins, M. E.; Spivak, D. A. *Anal. Chim. Acta* **2007**, *591*, 7.
3. Shi, X. Z.; Wu, A. B.; Qu, G. R.; Li, R. X.; Zhang, D. B. *Biomaterials* **2007**, *28*, 3741.

4. Zhang, T. L.; Liu, F.; Li, K. A. *Chem. J. Chin. Univ.* **2010**, *31*, 1126.
5. Huang, X. D.; Kong, L.; Li, X.; Zheng, C. J.; Zou, H. F. J. *Mol. Recognit.* **2003**, *16*, 406.
6. Takeuchi, T.; Minato, Y.; Takase, M.; Shinmori, H. *Tetrahedron Lett.* **2005**, *46*, 9025.
7. Beltran, A.; Borrull, F.; Cormack, P. A. G.; Marcé, R. M. *Trends Anal. Chem.* **2010**, *29*, 1363.
8. Dias, A. C. B.; Figueiredo, E. C.; Grassi, V.; Zagatto, E. A. G.; Arruda, M. A. Z. *Talanta* **2008**, *76*, 988.
9. Huang, Y. P.; Liu, Z. S.; Zheng, C.; Gao, R. Y. *Electrophoresis* **2009**, *30*, 155.
10. Ge, Y.; Turner, A. P. F. *Chem. Eur. J.* **2009**, *15*, 8100.
11. Pichon, V.; Chapuis-Hugon, F. *Anal. Chim. Acta* **2008**, *622*, 48.
12. Tokonami, S.; Shiigi, H.; Nagaoka, T. *Anal. Chim. Acta* **2009**, *641*, 7.
13. Pfister, J. R. J. *Nat. Prod.* **1988**, *51*, 969.
14. Albrecht, G.; Zundel, G. Z. *Naturforsch.* **1984**, *39A*, 986.
15. Sellergren, B.; Lepisto, M.; Mosbach, K. J. *Am. Chem. Soc.* **1988**, *110*, 5853.
16. Shea, K. J.; Spivak, D. A.; Sellergren, B. J. *Am. Chem. Soc.* **1993**, *115*, 3368.
17. Matsui, J.; Miyoshi, Y.; Doblhoff-Dier, O.; Takeuchi, T. *Anal. Chem.* **1995**, *67*, 4404.
18. Shi, R. X.; Guo, C. H.; Zou, X. H.; Zhu, C. Y.; Zhao, J. J.; Deng, Y. D. *Chin. Chem. Sens.* **2002**, *22*, 49.
19. Xu, Z. F.; Liu, L.; Deng, Q. Y. J. *Pharm. Biomed. Anal.* **2006**, *41*, 701.
20. Sellergren, B.; Shea, K. J. J. *Chromatogr. A* **1993**, *635*, 31.
21. Kugimiya, A.; Mukawa, T.; Takeuchi, T. *Analyst* **2001**, *126*, 772.
22. Rampey, A. M.; Umpleby, R. J.; Rushton, G. T.; Iseman, J. C.; Shah, R. N.; Shimizu, K. D. *Anal. Chem.* **2004**, *76*, 1123.
23. Cacho, C.; Schweitz, L.; Turiel, E.; Pérez-Conde, C. J. *Chromatogr. A* **2008**, *1179*, 216.
24. Zhang, T. L.; Meng, X. J.; Jia, J. F.; Han, L. L. *Chin. Anal. Chem.* **2010**, *38*, 33.
25. Zhang, Z. L.; Wang, B. J. *Appl. Polym. Sci.* **2009**, *113*, 1050.
26. Luliński, P.; Maciejewska, D. *Mater. Sci. Eng. C* **2011**, *31*, 281.