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Study on the mechanism of binding specificity of metoclopramide-imprinted polymers

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Abstract

A series of metoclopramide (MCP)-imprinted polymers utilizing methacrylic acid or 2-vinylprindine (2-VP) as functional monomer and chloroform, acetonitrile or methanol as porogen were prepared. The affinity and specificity of these polymers were evaluated by equilibrium binding experiments. Proton NMR model studies on interactions between the template and functional monomer analogues, acetic acid and d_5-pyridine, were performed in the same solvents that were used as porogens for the molecularly imprinted polymers (MIPs). A correlation was found to exist between the binding strength and specificity of a particular polymer and the extent of monomer–template interactions shown by the corresponding NMR spectrum. So, a useful means is provided to predict the performance of a MIP in this paper. Based on the results of NMR experiments and selectivity experiments, the role of functional groups of the template in the formation of complementary interacting sites in the polymer in different porogens was discussed, and the mechanism of molecular recognition of the MIPs was proposed.

Keywords: Molecular imprinting; Metoclopramide; Hydrogen bonding; NMR; Molecular recognition

1. Introduction

Molecular imprinting provides specific recognition sites for given molecules in synthetic polymers in tailor-made fashion. It has been drawing increasing attention in recent years, as MIPs have greater advantage such as predetermined selectivity, simple and convenient preparation, robustness in organic solvents and acidic or basic reagents, and durability to high temperature [1–8]. The principles of molecular imprinting are as follows: (1) complex formation between target molecule (template molecule) and functional monomer capable of interacting with the template molecule by covalent bonding and/or non-covalent bonding; (2) polymerization with a cross-linking agent; (3) removal of the template molecule from the cross-linked polymer, resulting in the formation of complementary binding sites for the template molecule. The functional groups of the resulting binding sites could be arranged at suitable positions for interaction with the template molecule.

The affinity and specificity of the polymers towards the template molecule depend on combined factors: the choice of the functional monomer(s), the solvent used as porogen [9] and the cross-linker, etc. It is often necessary to go through a lengthy process of experimental trial before finding the recipe to yield an imprint with a strong affinity and high specificity towards the template.

The presence of specific monomer–template interactions is the basis for template selective recognition sites in the resultant polymer. Interactions between functional groups of template and the functional monomer(s) can result in chemical shift changes on NMR spectrum [10–12]. So, a convenient way can be provided to study the extent of monomer–template interactions and to investigate the dependence of these interactions on solvent. This means that useful information for a more directed choice of functional monomer(s) and porogen can be obtained by NMR experiments [11]. In the present study, a series of MCP (Fig. 1) imprinted polymers were prepared in different porogens. The functional monomers used include methacrylic acid (MAA) and 2-vinylprindine (2-VP). The affinity and selectivity of these polymers were evaluated by equilibrium binding experiments. Proton NMR study model was used to study the interactions between the template and the functional monomer analogues in

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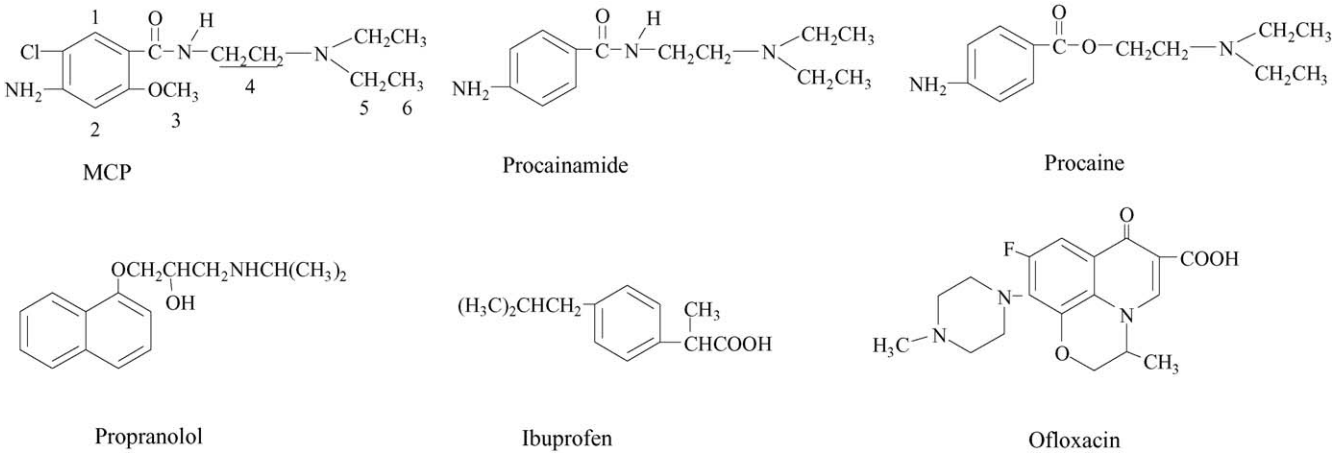

Fig. 1. Structures of the substrates used in this study and atomic labeling of MCP.

different solvents. Based on the results of NMR experiments and selectivity experiments, the role of the functional groups of the template in the formation of complementary interacting sites in the polymer in different porogens was discussed. Accordingly, the mechanism of molecular recognition of the MIPs was proposed.

2. Experimental

2.1. Materials

Ethylene dimethacrylate (EDMA) was obtained from Jiangsu Anli Chemical Plant. Before use, EDMA was distilled under vacuum after being extracted with 10% sodium hydroxide and dried over anhydrous magnesium sulfate. 2,2′-Azobis(isobutyronitrile) (AIBN) was obtained from Shanghai Chemical Plant (Shanghai, China) and recrystallized from methanol. MAA and 2-VP were purchased from Guangzhou Chemical Plant (Guangzhou, China) and Acros (NJ, USA), respectively. They were distilled under vacuum to remove the inhibitor before use. MCP was purchased from Yunpeng Medicinal Company (Shanxi, China). Procaine, procainamide and ofloxacin were obtained from Guangzhou South Hospital (Guangzhou, China). Ibuprofen was obtained from Shandong Xinhua Medical Plant (Shandong, China). Propranolol was obtained from Shantou Jinshi Medical Plant (Guangzhou, China). The other used reagents were d_5-pyridine (99 at.% D, Aldrich, USA), d_3-acetonitrile (99.8 at.% D, Cambridge Isotope Laboratories Inc., USA), d_4-methanol (99.5 at.% D, Chemalog Chemical Dynamics Corporation), and d-chloroform (≥99.8 at.% D, Aldrich, USA). All the solvents were analytical grade and used as received.

2.2. Preparation of the polymers

All the imprinted polymers (P_1–P_6) were prepared similarly with EDMA as the cross-linker. The molar ratio of cross-linker, functional monomer and template was 20:4:1. The procedure for the synthesis of the polymer P_1 is as follows: MCP (0.5 mmol) was dissolved with chloroform (6.0 ml) in a 50 ml glass ampoule, then MAA (2.0 mmol), EDMA (10.0 mmol) and AIBN (24.0 mg) were added. After nitrogen gas was sparged into the solution for 5 min, the ampoule was sealed under vacuum and the mixture was kept in a water bath at 60.0 °C for 24 h. The resultant rigid polymer was grounded and passed through a 90 μm sieve, fine particles were removed by repeated sedimentation in acetone. The obtained particles were soxhlet extracted with a mixture of methanol–acetic acid (9:1, v/v) for 72 h, then were washed with methanol to remove the residual acetic acid and dried to constant weight under vacuum at 60.0 °C. The reference non-imprinted polymers (NP_1–NP_6) were prepared using the same recipe as the corresponding MIP without addition of the template and worked up by the same procedure.

2.3. Binding experiments

Equilibrium binding experiments were conducted to evaluate the binding properties of the polymers. In the binding experiment of each polymer, the solvent used was the same as the porogen used for preparation of this polymer. The polymer particles (20.0 mg) were placed in a 10 ml conical flask and mixed with 3.0 ml of a known concentration of solution of selected substrate. The conical flask was shaken for 4.0 h at room temperature. Then the mixture was filtrated through a 0.45 μm filter. After that, 1.0 ml of the filtrate was used and diluted to 10.0 ml with addition of solvent. The concentration of the diluted solution was determined by a UV2100 spectrophotometer (Unico, Shanghai, China). The amount of substrate bound to the polymer (Q) was calculated according to

$$Q(\mu\text{mol}) = V(C_\text{i} - C_\text{l})$$

where V, C_i and C_l represent the volume of the solution (ml), initial solution concentration (μmol/ml) and the solution concentration after adsorption (μmol/ml), respectively. The specificity of the polymers was estimated by the distribution coefficients of substrates between polymer and solution. The distribution coefficient (K_d) is defined as [13]

$$K_\text{d}(\text{ml/g}) = C_\text{p}/C_\text{l}$$

where C_p is calculated according to

$$C_p\,(\mu\text{mol/g}) = \frac{Q\,(\mu\text{mol})}{\text{mass of polymer in g}}$$

2.4. NMR experiments

NMR spectra were obtained using a Mercury-plus 300 MHz spectrometer (Varian). The concentration of MCP in deuterated chloroform, acetonitrile or methanol was 0.03 mmol ml^{-1}, with 0.075 mmol of either acetic acid, or deuterated pyridine added.

3. Results and discussion

3.1. The specific affinity of the polymers for the template

In this work, in order to find out the suitable functional monomer and porogen and to produce a MIP that had a strong and highly specific affinity for the template, a series of MCP-imprinted polymers and corresponding reference non-imprinted polymers (NPs) were prepared using different monomers in different porogens. The amount of MCP bound to the MIPs and NPs were determined by equilibrium binding experiments. The obtained distribution coefficients of MCP are shown in Fig. 2.

The imprinted polymers used MAA as functional monomer (P_1–P_3) exhibited affinity and specificity for MCP. The polymers synthesized in chloroform (P_1) and acetonitrile (P_2) have much stronger binding affinity than those synthesized in methanol (P_3). The polymers with 2-VP as functional monomer (P_4–P_6) have almost no binding capacity for MCP.

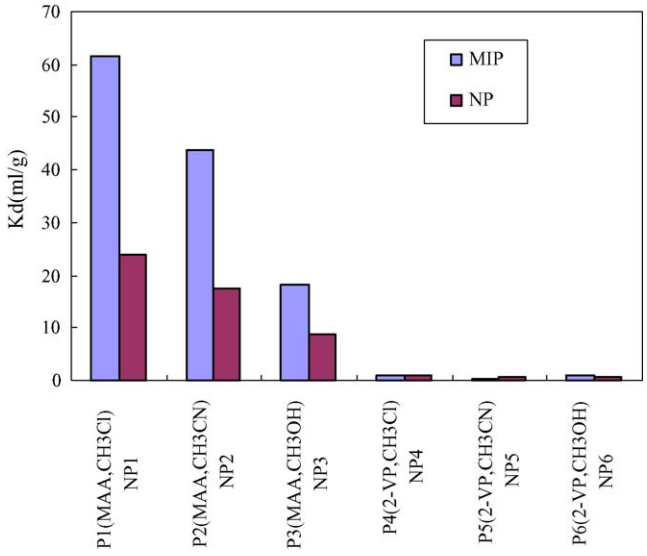

Fig. 2. K_d of MCP on MIPs and NPs prepared with different functional monomers and porogens under equilibrium binding conditions: initial concentration of MCP: 2.0 mmol/l; volume: 3.0 ml; adsorption time: 4 h.

3.2. Mechanism of binding specificity

3.2.1. ^1H NMR study

For non-covalent molecular imprinting, the complementary intermolecular interactions between template and functional monomers are critical factors for precise molecular recognition. NMR experiments can provide much useful information on the interactions between template and functional monomer(s). In general, the extent of the observed change of chemical shifts is proportional to the strength of the interactions. In this study, interactions between template and functional monomers are studied by proton NMR analysis. For the sake of simplicity, MAA and 2-VP were substituted by acetic acid and deuterated pyridine, respectively. Since the cross-linker and initiator have less influence on the interactions between template and functional monomers [14], the studies of NMR analysis were performed in the absence of EDMA and AIBN. The molar ratios of template and acetic acid or d_5-pyridine in the

Table 1
The change in NMR shift (Δ, ppm) of the metoclopramide protons in response to the addition of acetic acid or d_5-pyridine in different deuterated solvents

	1-CH	2-CH	3-CH_3	4-CH_2CH_2	5-CH_2	6-CH_3	NH_2	CONH
$CDCl_3$								
MCP	8.05	6.28	3.88	3.51	2.60	1.06	4.36	8.21
MCP–HAc	8.00	6.28	3.89	3.81	3.17	1.27	ND	8.31
Δ	−0.05	0	0.01	0.30	0.57	0.21	–	0.10
MCP–C_5D_5N	8.09	6.28	3.87	3.51	2.60	1.06	4.42	8.21
Δ	0.04	0	−0.01	0	0	0	0.06	0
CD_3CN								
MCP	7.85	6.47	3.86	3.36	2.53	1.01	4.85	8.11
MCP–HAc	7.82	6.46	3.87	3.60	3.05	1.18	4.97	8.28
Δ	−0.03	−0.01	0.01	0.24	0.52	0.17	0.12	0.17
MCP–C_5D_5N	7.86	6.47	3.86	3.36	2.53	1.01	4.88	8.12
Δ	0.01	0	0	0	0	0	0.03	0.01
CD_3OD								
MCP	7.80	6.47	3.89	2.65–3.45	2.62	1.07	3.30	ND
MCP–HAc	7.77	6.44	3.88	3.30–3.73	3.25	1.27	3.32	ND
Δ	−0.03	−0.03	−0.01	0.28–0.65	0.63	0.20	0.02	–
MCP–C_5D_5N	7.84	6.50	3.92	2.65–3.47	2.64	1.10	3.33	ND
Δ	0.04	0.03	0.03	0.02	0.02	0.03	0.03	–

ND, not detectable or not be distinguished.

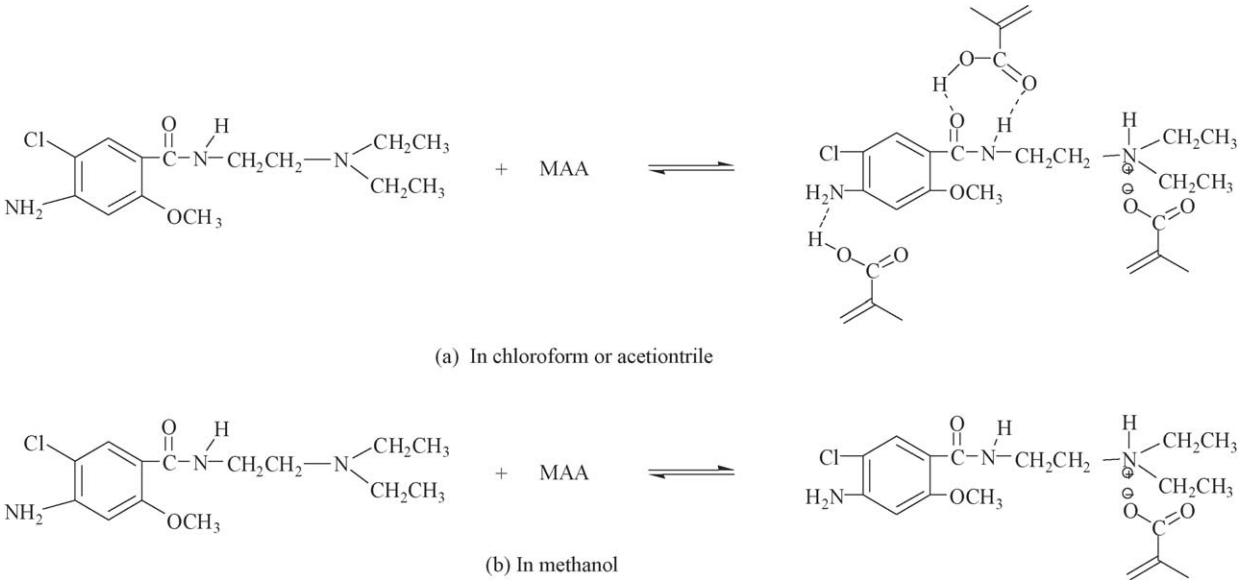

Fig. 3. Schematic illustration of the formation of the template–monomer complexes in different porogens.

NMR solutions were the same as those of template and functional monomer in the polymerization mixture. The effects of acetic acid or d_5-pyridine on the protons of MCP, taken from NMR spectra in $CDCl_3$, CD_3CN or CD_3OD, are shown in Table 1.

Currently, the carboxyl group is the most commonly used hydrogen bonding group in molecular imprinting, it can also form strong ionic interactions with basic functional groups. The addition of acetic acid to solution of MCP in $CDCl_3$, CD_3CN or CD_3OD, results in about 0.24–0.65 ppm shifts of a number of resonances arising from protons in the vicinity of the basic tertiary nitrogen. The relative large shift of the protons indicates that protonation of the tertiary amine and subsequent interaction, presumably by ion pairing with the carboxylate anion, results in a significant change in the magnetic environment of adjacent protons. When chloroform or acetonitrile was used as solvent, the chemical shifts of the protons of the amino group (NH_2) and amido group (CONH) were downfield in response to the addition of acetic acid (the NH_2 proton signal in the MCP-acetic acid in $CDCl_3$ cannot be distinguished). This provides clear evidence of hydrogen bonding between these two groups of MCP and carboxyl group of acetic acid. However, when methanol was used as solvent, the chemical shifts of the protons of NH_2 and CONH were observed to have little change. Methanol has strong hydrogen bonding ability and can efficiently compete with the functional monomer for the functional groups of the template. Thus, it can weaken or block the formation of hydrogen bonding between the functional monomer and the template. In the case of molecularly imprinted polymers prepared using non-covalent interactions (hydrogen bonding, ionic interactions, hydrophobic interactions, metal chelation, etc.), the extent of template complexation in the pre-polymerization mixture is a consequence of a series of equilibria [12]. When chloroform or acetonitrile was used as the solvents, the interactions between the template (MCP) and the monomer (MAA) include hydrogen bonding and ionic interactions (Fig. 3a). The hydrogen bonding strength is very much modulated by the medium, in the case of methanol used as the solvent, the hydrogen bonding between MCP and MAA does not exist or is very weak, ionic interaction is the primary interaction (Fig. 3b).

The data presented in Table 1 also showed that the chemical shift of the protons of MCP almost did not change when adding d_5-pyridine to the solution of MCP. This was suggestive of the absence of the interaction between pyridine and MCP, or the interaction is very weak.

Being a system in equilibrium, the stronger interactions between the template and the functional monomer(s), the more stable template–monomer complex being formed, which results to the increase in the concentration of the complex. This could be responsible for an increase in the number of specific binding sites created during polymerization. When 2-VP was used as the functional monomer, it cannot effectively interact with the template in the pre-polymerization mixture, thus the template–monomer complex is not formed, and the polymers (P_4–P_6) have no affinity for the template.

3.2.2. *Selectivity of the polymers*

The types of the complex formed in the pre-polymerization mixture are manifold, such as the template–monomer type illustrated in Fig. 3 and template–template–monomer type [12], etc. Some types of the complexes cannot survive the polymer synthesis step. In this study, it was found that the change of the chemical shift of the proton of the amido group (CONH) is very small (0.10 ppm) in chloroform after addition of acetic acid. We may doubt that the hydrogen bonding between the carboxyl group and the amido group is too weak to survive the polymer synthesis step and produces an interaction site in the cavity of the polymer (P_1). The selectivity tests of the polymers can show some evidence on this point.

The selectivity tests of P_1–P_3 and NP_1–NP_3 were carried out using a series of structurally related compounds (Fig. 1). Their amounts bound to the polymers were determined by equilibrium

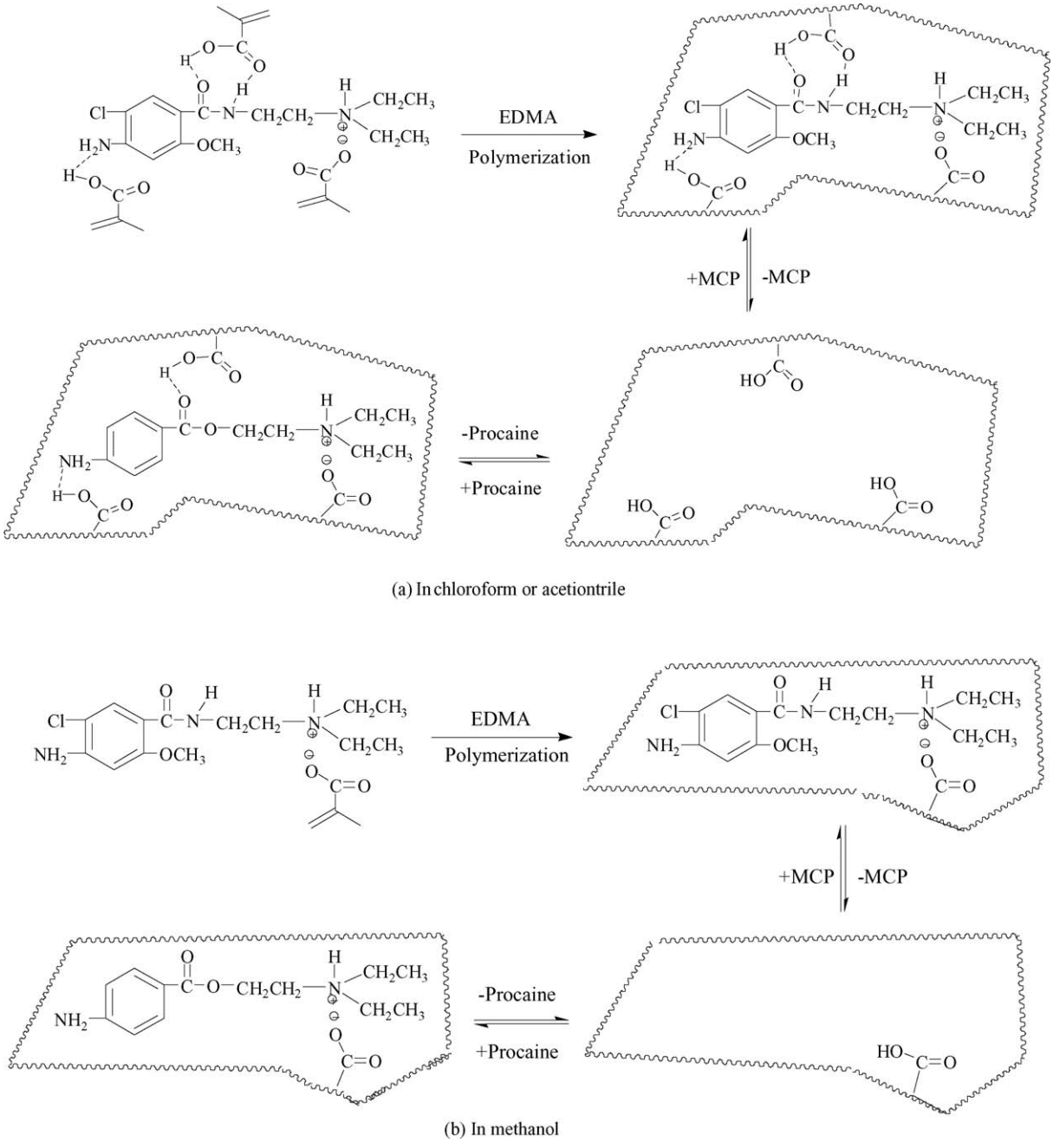

Fig. 4. The preparation process of MIPs and its interaction with procaine.

binding method. The distribution coefficients of the substrates were listed in Table 2.

The data in Table 2 shows that P_1 and P_2 exhibit high affinity for procainamide, the values of K_d are similar to that of the template. This could be easily explained by its close similarity to the template in the way of the arrangement of the functional groups and the size of the three-dimensional structure. Procainamide can enter the microcavity based on shape selection and position of functional groups created by the imprinting method. For procaine, the structural difference is an ester group instead of an amido group as in procainamide, however, P_1 and P_2 show lower affinity for it than that of procainamide and MCP. This indicates that there are three carboxylic residues, cre-

Table 2
K_d (ml/g) of the structurally related compounds on MIPs and NPs prepared in different porogens under equilibrium binding conditions

	P_1	NP_1	P_2	NP_2	P_3	NP_3
MCP	61.54	23.94	43.62	17.60	18.21	8.85
Procainamide	64.63	26.21	48.08	20.44	19.78	10.56
Procaine	51.38	20.74	38.76	14.52	20.35	9.17
Proranolol	5.44	1.66	8.34	7.61	1.34	0.67
Ibuprofen	0	0	1.26	2.30	0	0
Ofloxacin	1.78	3.65	4.77	4.86	2.15	1.22

Initial concentration of the substrates: 2.0 mmol^{-1}; volume: 3.0 ml; adsorption time: 4.0 h.

ated during the imprinting process by the functional groups of the template, i.e. amino group, amido group and tertiary amine group, in the microcavities in P_1 and P_2 (Fig. 4a). When procainamide enters in the cavity, its three functional groups can interact with the three carboxylic residues, respectively. Since an ester group has a weaker hydrogen bonding ability than an amido group, this results in a lower K_d value for P_1 and P_2 for procaine compared with procainamide and MCP (Fig. 4a). P_3 exhibited much lower affinity for MCP, procainamide and procaine, because there is only one carboxylic residue created by the tertiary amine group in the microcavities of P_3 (Fig. 4b). P_3 adsorbs these substrates through electrostatic interactions in methanol. Because the sizes of the three-dimensional structures of the other tested substrates are dissimilar, the MIPs bind them through weak non-specific adsorption and do not show any selectivity for them. Compared with the imprinted polymers, the data of non-imprinted polymers (NP_1–NP_3) shows that these polymers have considerably less binding for the selected substrates (Table 2). This suggest the existence of cavities in MIPs which are complementary both in shape and the functional group arrangement to the template molecule, and that the binding ability is introduced into the MIPs by the molecular imprinting technique.

4. Conclusions

In this study, a series of MCP-imprinted polymers were prepared using MAA or 2-VP as the functional monomers and chloroform, acetonitrile or methanol as the porogens. All of the MIPs using MAA as the functional monomer exhibited affinity and specificity for MCP, among which the polymers synthesized in chloroform (P_1) and acetonitrile (P_2) have much stronger binding affinity than the polymers synthesized in methanol (P_3). The proton NMR studies indicate that, in chloroform or acetonitrile, the interactions between MAA and MCP include hydrogen bonding and ionic interactions, the templates and the functional monomers can form stable complexes in the pre-polymerization mixture. There are three carboxylic residues, created during the imprinting process by the functional groups of the template, arranging at suitable positions in the microcavities of P_1 and P_2. When methanol was used as the solvent, the main interactions between MAA and MCP are ionic interactions, the complexes formed in the pre-polymerization mixture are not so stable as those in chloroform or acetonitrile. There is only one carboxylic residue in the microcavities of P_3 created by the tertiary amine group of MCP through imprinting. When 2-VP was used as the functional monomer, it cannot effectively interact with the template in the pre-polymerization mixture, thus the template–monomer complex is not formed, and the polymers (P_4–P_6) have no affinity for the template. The study reveals that the proton NMR studies can provide useful information for us to understand the mechanism of molecular recognition and a more directed choice of MIP ingredients, which in turn should help us to shorten the process of making MIPs.

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