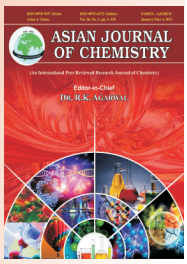
Asian Journal of Chemistry; Vol. 26, No. 18 (2014), 6039-6042

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16542



# Preparation of Molecularly Imprinted Polymer and Its Recognition Property for Acetanilide

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*Received*: 17 October 2013; *Accepted*: 3 April 2014; *Published online*: 1 September 2014; AJC-15849

Molecularly imprinted polymers with highly selective recognition for acetanilide were prepared by precipitation polymerization using acetanilide as template molecule, methacrylic acid as functional monomer and ethylene glycol dimethaerylate as crosslinker. The experiment measured the influence of the molar ratio of the template molecule and the functional monomer upon the adsorption capacity of the polymers and the optimized ratio was obtained to be *n*(acetanilide) : *n*(methacrylic acid) = 1:4. Scatchard analysis indicated the imprinted polymer acetanilide template molecule and functional monomer methacrylic acid formed one kind of binding site, binding site dissociation constant $K_D$ = 4.35 mmol/L, the apparent maximum binding capacity $Q_{max}$ = 111.87 μmol/g.

**Keywords: Acetanilide, Molecularly imprintied polymer, Adsorption property, Spectrometric detection.**

## INTRODUCTION

Molecularly imprinted polymers (MIPs) are tailor-made porous materials with specific affinity and high selectivity for a particular target molecule[1,2]. These polymers are commonly prepared with polymerizable functional monomers and crosslinkers that are surrounded around a template molecule. After polymerization, template molecules are removed from the polymer matrix to form vacant imprinted sites that are complementary in shape, size and functionalities to those of the template[3]. Molecularly imprinted polymers are tolerance to a wide range of pH, high pressure, elevated temperature, relative ease and low cost of preparation and reusability[4,5]. These outstanding advantages make molecularly imprinted polymers ideal for extensive application in solid-phase extraction[6,7], sensors[8,9], drug delivery[10], chromatography[11] and catalysis[12].

Acetanilide, a raw material for sulfa drugs, can be used to make analgesics, antipyretics, preservatives or dye intermediates. Meanwhile, it is a highly toxic organic substance which can be extremely harmful to human health if being inhaled or eaten accidentally. It is irritating to the upper respiratory tract and skin and thus may result in dermatitis. A high intake of acetanilide may lead to methemoglobinemia and myeloproliferative diseases. Acetanilide can suppress the central nervous system and cardiovascular system of human body and exposure to large amount of it may induce symptoms including dizziness and pale complexion.

In the present work, molecularly imprinted polymers with special molecule recognition properties for acetanilide have been successfully prepared by precipitation polymerization using acetanilide as template molecule, methacrylic acid as functional monomer and ethylene glycol dimethacrylate as crosslinker.

## EXPERIMENTAL

Antifebrin, aniline, acetaminophen and *p*-anisidine were purchased from Shanghai Chemical Reagent Co., Ltd., Shanghai, China. Azodiisobutyronitrile (AIBN) was purchased from Tianjin Chemical Engineering Institute, Tianjin, China. Acetonitrile was purchased from Tianjin Kermel Chemical Reagent Co., Ltd., Tianjin, China. Acetic acid was purchased from Tianjin chemical plant, Tianjin, China. Methanol was purchased from Sinopharm Group Chemical Reagent Co., Ltd. Unless specially stated, all reagents used were of analytical grade.

A model T6 UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China) was used for recording the absorption spectra and photometric measurements. The static experiments of adsorption were carried out at a certain temperature in a model DKZ-1 Oscillating Water Tunnel (Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China). A model TDL80-2B centrifuge (Shanghai Anting Scientific Instrument, Shanghai, China) was used for separating substances.

**Molecularly imprinted polymer preparation:** Precipitation polymerization was conducted and the procedures were as follows: 0.2703 g (2 mmol) acetanilide was dissolved in 70 mL acetonitrile with the corresponding ratio of methacrylic acid. The solution was kept for 4 h after being in a sonicating bath for 20 min to facilitate the sufficient interaction of the template molecules and the functional monomers. Then, 7.80 mL (40 mmol) ethylene glycol dimethacrylate and 50 mg azodiisobutyronitrile were added. The mixture was sonicated for another 20 min in order to be dissolved and mixed perfectly and sparged with $N_2$ for 15 min. Subsequently, the solution was sealed and immersed at 60 °C in the thermostat for 24 h until white lump polymers were formed. The polymers were ground and filtered through 75 μm sieve. The method of acetone sedimentation was also utilized to rid of undersized particles. The particles collected were then placed in Soxhlet extraction, extracted with $CH_3OH/CH_3CO_2H$ (v/v, 9/1) until the acetanilide cannot be detected. The stage was monitored by a UV-visible spectrophotometer. Afterwards, the polymers was washed by methanol for washing out the acetic acid. Last, they were dried inside a vacuum oven at 55 °C for 24 h, after which the acetanilide molecularly imprinted polymers with methacrylic acid as the functional monomer were obtained.

Non-imprinted polymers (NIPs) were prepared *via* the same procedure in the absence of template molecule acetanilide.

**Adsorption assay:** 20 mg of each of non-imprinted polymer and molecularly imprinted polymer that prepared from different ratios of template to monomer were added into 3 mmol/L acetanilide in acetonitrile solution in glass tubes, which would be stirred at room temperature for 12 h. Then the mixture was filtered and the filtrate collected, the absorbance of which was measured at 241 nm with a UV-visible spectrophotometer. Based on the concentration change before and after the adsorption, the adsorption capacity Q of the polymers was calculated according to eqn. 1:

$$Q = (C_0\text{-}C)V/W \quad (1)$$

where, $C_0$ (mmol/L) is the initial concentration of acetanilide; C (mmol/L) is the final concentration of acetanilide after adsorption; V (mL) is the volume of the solution; W (g) is the weight of the polymers used. All experiments were carried out 3 times and the mean values were used in data.

**Studies on adsorption property of polymers:** 20 mg of molecularly imprinted polymers (template molecule/functional monomer, 1/4) or non-imprinted polymers were suspended in 5 mL acetanilide in acetonitrile solution with an initial concentration of 3 mmol/L, which were stirred at room temperature for 12 h in accordance with the procedure mentioned in adsorption assay. The adsorption amount was calculated and adsorption isotherms drawn for Scatchard analysis.

**Studies on kinetics of imprinted polymers:** Molecularly imprinted polymer was chosen to further the studies on adsorption kinetics of the imprinted polymers. In each test, 20 mg of molecularly imprinted polymer was precisely prepared and their adsorption of acetanilide was measured at regular intervals.

**Selective adsorption of imprinted polymers:** Aniline, acetaminophen and *p*-anisidine, the structures of which are similar to that of acetanilide, were selected as substrates and added into 3 mmol/L acetonitrile solution, respectively. In accordance with static adsorption method, the equilibrium adsorption capacity of each substrate on molecularly imprinted polymer and non-imprinted polymer can be calculated based on the concentration change of the substrates before and after the adsorption.

## RESULTS AND DISCUSSION

**Spectrophotometric analysis of interaction between acetanilide and methacrylic acid:** Methacrylic acid was chosen as functional monomer and the concentration of acetanilide in acetonitrile solution was fixed at 0.05 mmol/L. Different amounts of functional monomers were added each time to make the molar ratio of template molecule to functional monomer 1:0, 1:2, 1:4, 1:6 and 1:8, respectively. The ultraviolet adsorption spectra of acetanilide were shown in Fig. 1.

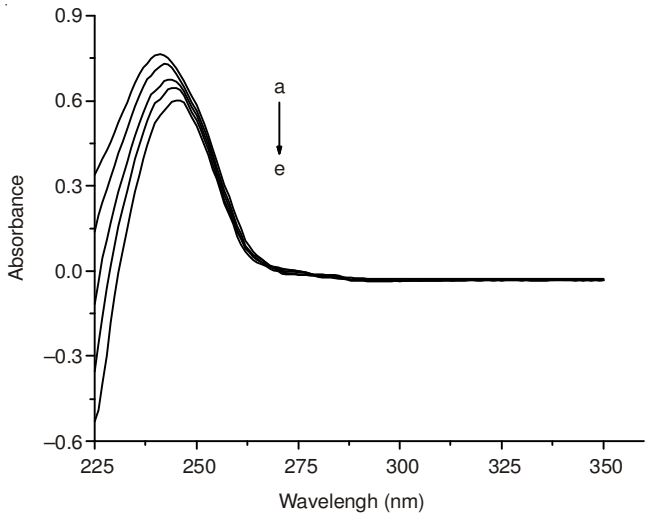


Fig. 1. UV absorption spectrum of acetanilide to different molar ratio of methacrylic acid. Curves a-e: stand for acetanilide to methacrylic acid from 1:0, 1:2, 1:4, 1:6, 1:8, in molar ratio, respectively

As shown in Fig. 1, when adding methacrylic acid into acetanilide in acetonitrile solution, as the concentration of the functional monomer increased, red shift of spectra was witnessed, which indicated that the template molecule and functional monomer interacted with each other and generated a compound[13].

**Optimization of molar ratio of functional monomers and the template molecules:** With the template molecule and the cross-linker fixed, when different amounts of methacrylic acid were added, the experiment measured the influence of the molar ratio of the template molecule to the functional monomer upon the adsorption capacity of the polymers, as shown in Table-1. In order to compare imprinting effects, the imprinting factor (IF), selectivity (α) were calculated as in eqns. (2) and (3)[14]:

$$IF = Q_{MIP}/Q_{NIP} \quad (2)$$

$$\alpha = (Q_{MIP}\text{-}Q_{NIP})/Q_{NIP} \quad (3)$$

where: $Q_{MIP}$ is the adsorption capacity of the imprinted polymers; $Q_{NIP}$ is the adsorption capacity of non-imprinted polymers.

As shown in Table-1, when the molar ratio of the template molecule to the functional monomer methacrylic acid was 1:4, the prepared molecularly imprinted polymers had relatively large adsorption capacity and the best imprinting effect. When the ratio was higher (*i.e.*, 1:2), the molecular imprinting of the template was not sufficient, which resulted in small adsorption capacity. When it was lower (*i.e.*, 1:6 or 1:8), excessive functional monomer led to an increase in non-specificity recognition sites in the polymers, which in turn reduced the selectivity of the imprinting. Therefore, the molar ratio of the template molecule and the functional monomer should be 1:4 according to the experiment.

TABLE-1
OPTIMIZATION OF MOLAR RATIO OF THE FUNCTIONAL MONOMERS AND THE TEMPLATE MOLECULES

| Polymers | Molar ratio of templates to monomers in molecularly imprinted polymer | $Q_{MIP}$ (μ mol/g) | $Q_{NIP}$ (μ mol/g) | IF | α |
|---|---|---|---|---|---|
| MIP1 | 1:2 | 32.51 | 29.87 | 1.09 | 0.09 |
| MIP2 | 1:4 | 44.15 | 15.76 | 2.80 | 1.80 |
| MIP3 | 1:6 | 49.54 | 38.27 | 1.29 | 0.29 |
| MIP4 | 1:8 | 30.36 | 27.43 | 1.11 | 0.11 |

**Adsorption properties of imprinted polymers and scatchard analysis:** Static adsorption method was applied to observe the adsorption of acetanilide on molecularly imprinted polymer and non-imprinted polymer with the concentration of antifebrin ranging from 0.25 to 3 mmol/L. The adsorption isotherms of acetanilide on molecularly imprinted polymer and non-imprinted polymer were drawn after being measured by the UV-visible spectrophotometer (Fig. 2). Within the concentration range measured, both molecularly imprinted polymer and non-imprinted polymer adsorbed certain amounts of acetanilide. The adsorption capacity of molecularly imprinted polymer was obviously higher than that of non-imprinted polymer and the gap widened with increasing concentration of the solution. The result indicated that molecularly imprinted polymer had specific recognition capacity of acetanilide and achieved adsorption through hydrogen bonding and space matching. Without the binding sites for specific selectivity, non-imprinted polymer mainly relied on the non-specific adsorption of its surface and thus had weaker adsorption capacity.

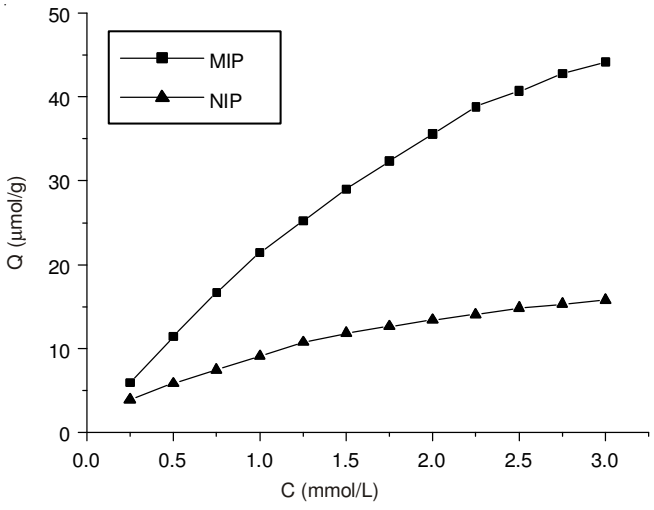


Fig. 2. Adsorption isotherms of molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP)

The Scatchard isotherm model, which is commonly used to evaluate the binding properties of imprinted polymers[5,7]. Scatchard equation is as follows:

$$Q/[C] = (Q_{max} - Q)/K_D \quad (4)$$

where Q (μmol/g) is the adsorption capacity of acetanilide, $Q_{max}$ (μmol/g) is the apparent maximum number of binding sites, $K_D$ (mmol/L) is the equilibrium dissociation constant and [C] (mmol/L) is the equilibrium concentration of acetanilide. The chart was drawn using Q as the X axis and Q/[C] as the Y axis.

As shown in Fig. 3, the Scatchard plot shows a linear relationship. It indicated that only a single type of binding site was formed in the acetanilide imprinted polymers under the studied concentrations. The linear regression equation is Q/[C] = 25.73-0.23 Q. According to the slope and intercept of the equation, $K_D$ and $Q_{max}$ were calculated to 4.35 mmol/L and 111.87 μmol/g.

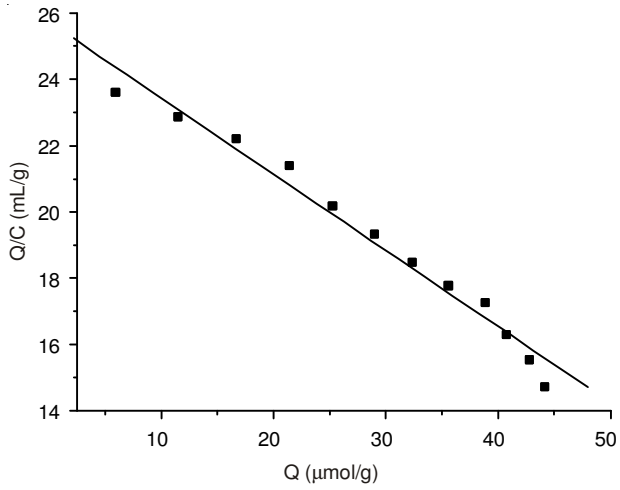


Fig. 3. Scatchard plot of molecularly imprinted polymer

**Adsorption kinetic curves of molecularly imprinted polymers:** The acetonitrile solution of acetanilide was prepared and diluted to 2 mmol/L and molecularly imprinted polymer's adsorption capacity of acetanilide was measured at different time points. It can be seen from Fig. 4 that the imprinted polymers' adsorption capacity of resorcinol incremented remarkably within the first 3 h, then increased slowly and gradually reached equilibrium after 6 h. The reason was that the adsorption initially took place at the shallow cavities on the polymers. The template molecules were captured by the imprinting cavities on the surface of the polymers quickly, resulting in fast adsorption speed. After the cavities were saturated, the template molecules began to penetrate the polymers to the binding sites inside them. Due to steric hindrance effect during mass transfer of the template molecules to the deeper cavities, the binding between the template molecules and the inner binding sites was deferred and the acceleration of the adsorption became negative with the lapse of time. The result proved the specific memory effect of the imprinted polymers on acetanilide molecules.

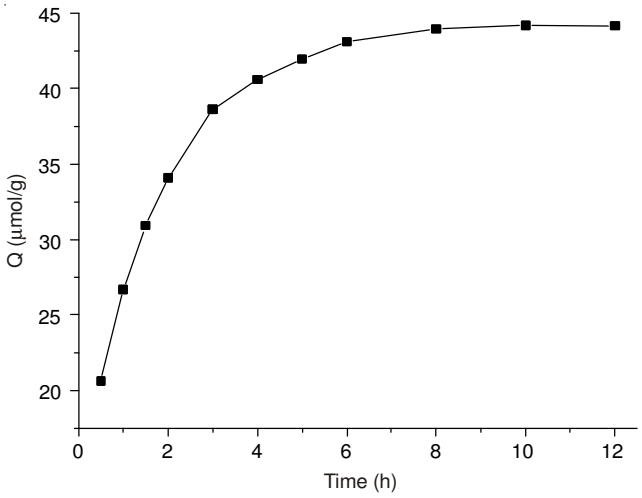


Fig. 4. Adsorption kinetics curve of molecularly imprinted polymer

**Recognition properties of imprinted polymers on different substrates:** To identify the recognition properties of the imprinted polymers on different substrates, aniline, acetaminophen and *p*-anisidine, the structures of which are similar to that of acetanilide, were selected (Fig. 5) for the adsorption static equilibrium experiment. The binding capacities of molecularly imprinted polymer and non-imprinted polymer with different substrates were demonstrated in Table-2.

TABLE-2
ADSORPTION AMOUNTS AND SELECTIVITY FACTORS OF MOLECULARLY IMPRINTED POLYMER AND NON-IMPRINTED POLYMER FOR DIFFERENT SUBSTRATES

| Substrate | $Q_{MIP}$ (μmol/g) | $Q_{NIP}$ (μmol/g) | IF | α |
|---|---|---|---|---|
| Acetanilide | 44.15 | 15.76 | 2.80 | 1.80 |
| Aniline | 26.32 | 22.97 | 1.15 | 0.15 |
| Acetaminophen | 10.73 | 8.29 | 1.29 | 0.29 |
| *p*-Anisidine | 8.94 | 7.02 | 1.27 | 0.27 |

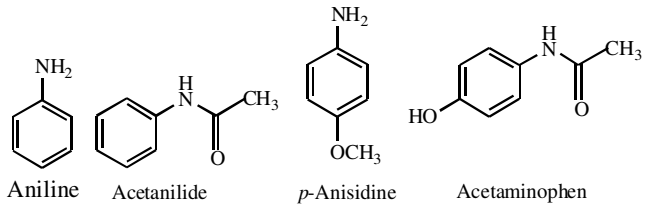


Fig. 5. Structures of analogues

As shown in Table-2, among the analogues, the adsorption amount of aniline is the most, with acetaminophen ranking the second and *p*-anisidine the weakest, but all less than that of acetanilide. On the basis of the fact, a conclusion can be drawn that molecularly imprinted polymer's imprinting cavities structurally matched well with the template molecule acetanilide and thus demonstrated the highest adsorption capacity and specific adsorption selectivity.

Remarkably, molecularly imprinted polymer's recognition of substrates depends on not only its structural matching with the substrates, but also interactions between the functional monomers of the polymers and the substrates. Therefore, the type and the amount of the functional monomers are highly relevant to the adsorption selectivity of molecularly imprinted polymer. The weakly acid functional monomer methacrylic acid used in the experiments had both strong hydrogen bonding with the template molecule acetanilide and weak electrostatic interaction with the alkaline templates, both facilitating the generation of the template monomer complex and the mutual recognition of molecules, which was echoed by molecularly imprinted polymer's selective recognition of different substrates.

With regard to the three analogues, the polymers' adsorption capacity of aniline was the highest, ascribable to small volumes of the aniline molecules, enabling them to enter the cavities of the polymers more easily. However, molecularly imprinted polymer's adsorption capacity of aniline did not differ significantly from non-imprinted polymer's, meaning no specific adsorption was demonstrated. As for acetaminophen and *p*-anisidine, since they did not fit the cavities and the functional groups of the imprinted polymers, the adsorption capacities of both of them were low.

## Conclusion

In this study, molecularly imprinted polymers for recognizing acetanilide were prepared by precipitation polymerization using acetanilide as template molecule, methacrylic acid as functional monomer and ethylene glycol dimethacrylate as crosslinker. The study indicated that the molecularly imprinted polymers had remarkably adsorption property and selectivity toward acetanilide. The paper laid a solid theoretical foundation for separation and detection of acetanilide imprinted polymers from environment and food.

## ACKNOWLEDGEMENTS

This work was supported by Foundation and Research in Cutting-Edge Technologies of Henan Province (No. 132300-413209).

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