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Synthesis and Study of Guest-Rebinding of MIP Based on MAA Prepared using Theophylline Template

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Abstract. A molecularly imprinted polymer (MIP) based on methacrylic acid (MAA) monomer and theophylline template has been synthesized using a modified bulk polymerization method. Theophylline was employed as a template and it formed a complex with MAA through hydrogen bonding. Self-assembly of template-monomer was followed by cross-linking process using ethylene glycol dimethacrylate (EGDMA) cross-linker. The polymerization process was initiated by thermal decomposition of benzoyl peroxide (BPO) as the initiator at 60^{o}C after cooling treatment at -5^{o}C. After 7 hours, a rigid polymer was obtained and followed by grinding the polymer and removing the template. As a reference, a non-imprinted polymer (NIP) has also been synthesized using similar procedure by excluding the template. FTIR study was carried out to investigate the presence of theophylline in the as-prepared polymer, MIP, and NIP. The spectra indicated that theophylline was successfully incorporated in the as-prepared polymer. This result was also confirmed by EDS analysis showing that N atoms of the as-prepared polymer were derived from amino group of theophylline. Furthermore, the polymer particles of MIP were irregular in shape and size as shown by its SEM image. The capability of guest-rebinding of the MIP was analyzed through Batchwise guest-binding experiment. The results showed that for initial concentration of theophylline in methanol/chloroform (1/1, v/v) of 0.333 mM, the binding capacity of the MIP was 23.22 μmol/g. Compared to the MIP, the adsorption capacity of the NIP was only 3.73 μmol/g. This result shows that MIP has higher affinity than NIP.

1. Introduction

Theophylline (1.3-dimethylxanthine) is a member of methylxanthine groups of drugs used in the treatment of pulmonary diseases such as asthma, neonatal apnea, and chronic obstructive pulmonary disease (COPD) [1, 2, 3]. In the pulmonary system, theophylline acts as bronchodilator by relaxing smooth muscle in the bronchial airways and pulmonary blood vessels [1, 4]. However, theophylline has a narrow therapeutic index [4]. Too high concentration of theophylline in plasma could cause toxicity or permanent neurological damage [3]. Plasma concentration of 25 μg/ml may lead to convulsion and death, while a rare effect such as seizure may occur at plasma concentration below 40

μg/ml [1]. Theophylline level in the blood serum is affected by age, food, genetic, and type of disease [1]. Therefore, it is required to monitor the theophylline level in the blood serum.

Researchers have developed molecularly imprinted polymers (MIPs) as a promising method in sensor technology due to its simplicity and low cost of fabrication [3]. MIPs have great potential applications in many fields such as antibody mimic for drug assay [5], drug delivery, signaling polymers, catalyst, chromatographic applications, herbicide detecting analysis [6], solid phase extraction (SPE) in milk [7], SPE for theophylline extraction from human serum [8], etc.

MIP is an artificial receptor that utilizes networks in the polymer to store memory of the imprinted molecule or template. It is prepared by polymerizing a monomer that is co-polymerized with a cross-linking agent in the presence of an imprint molecule [6]. In a non-covalent imprinting, template molecules bind to the functional residue derived from monomer through non-covalent interaction such as electronic interaction, hydrophobic interaction, hydrogen bonding, etc. [3]. Specific cavities are produced in the MIP after template extraction to provide guest-binding sites [6].

Polymerization process holds an important key in obtaining an MIP with good binding capacity. Bulk polymerization method is one of the most used polymerization methods because of its simplicity and no requirement of any sophisticated reagents and equipment [9]. Vlatakis, et al. [5] has produced theophylline MIP based on MAA for antibody mimic in drug assay that was polymerized at 60^{o}C for 24 hours. Similar polymerization conditions have also been used in obtaining theophylline MIP polymerized in oil bath [8] or water bath [10, 11]. However, these methods were quite time consuming.

In this study, a modified bulk polymerization method has been conducted in obtaining a good MIP with higher binding affinity compared to a non-imprinted polymer (NIP). The polymerization process was carried out after cooling treatment of the pre-polymerization mixture. Using this method, the overall time of MIP preparation was reduced while maintaining the simplicity of the former method.

2. Experimental Section

2.1. Materials

Theophylline anhydrous, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and benzoyl peroxide (BPO) were purchased from Sigma. Methanol, acetic acid, and chloroform were from Merck. All of the reagents were analytical grades and used as supplied without further purification.

2.2. Preparation of Molecularly Imprinted Polymer

The molecularly imprinted polymer (MIP) was prepared using a modified bulk polymerization method by including cooling treatment in the polymerization process [12, 13]. Theophylline was used as template molecules and imprinted in a polymer matrix of MAA functional monomer that was co-polymerized with EGDMA cross-linker. In a typical synthesis, into 2.5 mL of chloroform, 0.045 g of theophylline was dissolved under magnetic stirring. After that, 0.1 mL of MAA, 0.6 mL of EGDMA, and 0.05 g of BPO were added into the mixture and stirred for 20 min. For complete dissolution of the solids, the mixture was placed in an ultrasonic bath for 45 min. The mixture was then divided into some vials and loaded into a freezer at -5^{o}C for 1 h. Free radical polymerization was initiated by thermal decomposition of BPO as the initiator after the mixture was placed in an oven at 60^{o}C. A rigid polymer called as an as-prepared polymer was obtained after 7 h of polymerization process and it was then ground using a mechanical mortar. As a reference, a non-imprinted polymer (NIP) was also synthesized using a similar procedure without the presence of theophylline.

2.3. Template Molecules Removal

In order to remove theophylline from the as-prepared polymer matrix, 0.2 g of the as-prepared polymer was treated by repetitive sedimentation in 3 mL of acetonitrile for 20 h and then extensively washed using methanol/acetic acid (9/1, v/v) by sonicating the mixture for 1 h. The template removal process was repeated several times in order to obtain a free-template polymer or an MIP. Finally, the wet MIP was dried in air. For analysis purpose, the NIP was also treated in a similar manner.

Chemical bonding of the as-prepared polymer, MIP, and NIP were characterized using a Fourier transform infrared (FTIR) spectrometer (Bruker ATR FTIR Alpha) over the 4000-500 cm^{-1} range and at a resolution of 4 cm^{-1}. The polymer was also characterized using a scanning electron microscope (SEM JEOL JSM-6510LV) and energy-dispersive X-ray spectroscopy (EDS) for morphology and elemental analysis, respectively.

2.4. Batchwise Guest-Binding Experiment

To study the binding capacity of the MIP, Batchwise guest-binding experiment was carried out by incubating the MIP in theophylline solution. A 0.333 mM of theophylline solution was prepared by dissolving some amount of theophylline in 10 mL of methanol/chloroform (1/1, v/v). Into this solution, 20 mg of the MIP was added and the mixture was incubated for 14 h under magnetic stirring. After that, the mixture was centrifuged at about 1200 rpm for 15 min. The final concentration of theophylline in the supernatant was measured using UV spectrometer (Agilent 8453) at a wavelength of 272 nm. The amount of theophylline bound to the MIP (B_{bound}) was calculated by subtracting the final concentration of theophylline ($B_{unbound}$) to its initial concentration (C) as shown by Eq. (1).

$$B_{bound} = (C - B_{unbound}) \times \frac{V}{M} \tag{1}$$

where C and $B_{unbound}$ are in mol/L, V is in mL (volume of the solution), M is in mg (mass of the MIP), and B_{bound} is in mol/g. As a reference, a similar Batchwise guest-binding procedure was also applied to the NIP as the guest-binding agent.

3. Results and Discussion

3.1. Preparation of Molecularly Imprinted Polymer

Figure 1 shows the schematic illustration of imprinting process of theophylline in MAA-based polymer matrix. MAA, with its carboxylic acid group, has been chosen as the monomer so that it could be bound to amino group of theophylline through hydrogen bonding (O-H and/or N-H). Self-assembly between theophylline and MAA formed a template-monomer complex that undergoes polymerization in the presence of EGDMA cross-linker. The cross-linker provides rigid structure of the polymer and, with similar reactivity of EGDMA and MAA, enables the functional residue to uniformly distribute in the polymer network [6]. Chloroform, a non-polar porogenic solvent, was employed in the polymerization process due to its capability in dissolving all of the precursors and provides porous structure to the as-prepared polymer.

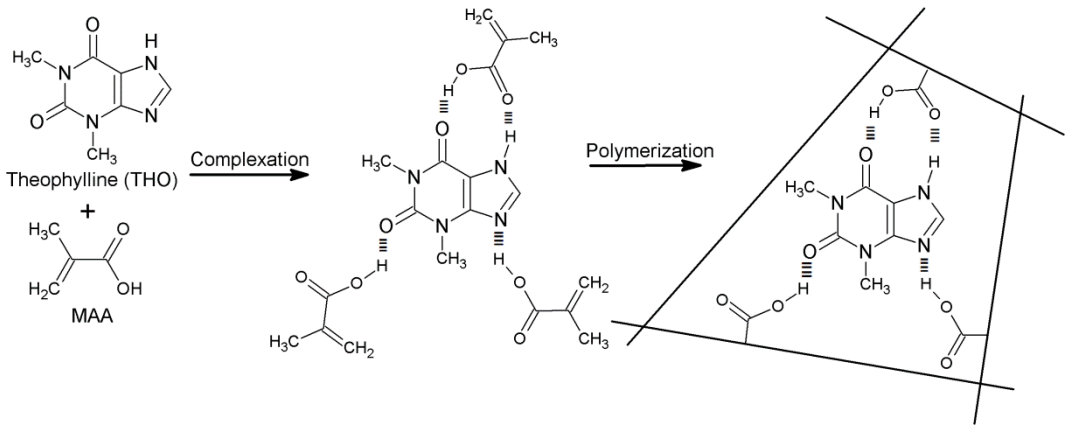

FIGURE 1. Schematic illustration of theophylline imprinting in MAA monomer co-polymerized with EGDMA cross-linker [6]

Figure 2 shows FTIR spectra of theophylline, as-prepared polymer, MIP, and NIP. Theophylline, with its amino group (Fig. 1), shows a broad and weak transmission band of N-H stretching vibration that centered at 3000 cm^{-1}. C-H stretching vibrations superimposed upon the N-H broad bend at 3054 and 2983 cm^{-1}. Besides that, N-H bending and stretching vibrations were observed at 1663 and 1561 cm^{-1}, respectively. Furthermore, carbonyl group (C=O) of theophylline was observed at 1705 cm^{-1} with medium transmission intensity.

Transmission band with strong intensity at 1716 cm^{-1} were observed in the FTIR spectra of the as-prepared polymer (Fig. 2.(b)) indicating C=O stretching vibrations from the carboxylic acid group of MAA. Moreover, O-H bonding of carboxylic acid group of MAA was found as bending vibrations at 1450 cm^{-1} with medium

intensity. Theophylline as the template molecules was successfully incorporated in the as-prepared polymer as shown by the appearance of transmission bands at 1670 and 1566 cm^{-1}. These bands were respectively associated with N-H bending and stretching vibrations from amino group of theophylline. These vibration modes usually appear in the range of 1650-1580 cm^{-1} and slightly shift to higher frequencies in the intermolecular bonding [14]. Compared to the as-prepared polymer, the FTIR spectra of the NIP (Fig. 2.(d)) do not show any bands associated with theophylline as a result of the absence of theophylline in its preparation.

An EDS spectrum of the as-prepared polymer is given by Fig. 3.(a). The spectrum shows that the as-prepared polymer contains C, N, and O atoms with mass percentage of 47.59, 38.90, and 9.01%, respectively. While for the NIP, its EDS spectrum (Fig. 3.(b)) does not show any contents of N atom, with mass percentage of C and O atoms of 82.98 and 17.02%, respectively. As from the FTIR spectra analysis, these results also indicate that the theophylline was incorporated in the as-prepared polymer. For morphology analysis, Fig. 3.(d) gives an SEM image of the MIP. The image shows that the polymer particles were irregular in shape and size. This result was the effect of grinding process of the monolith polymer in the preparation process of the MIP employing bulk polymerization method [9].

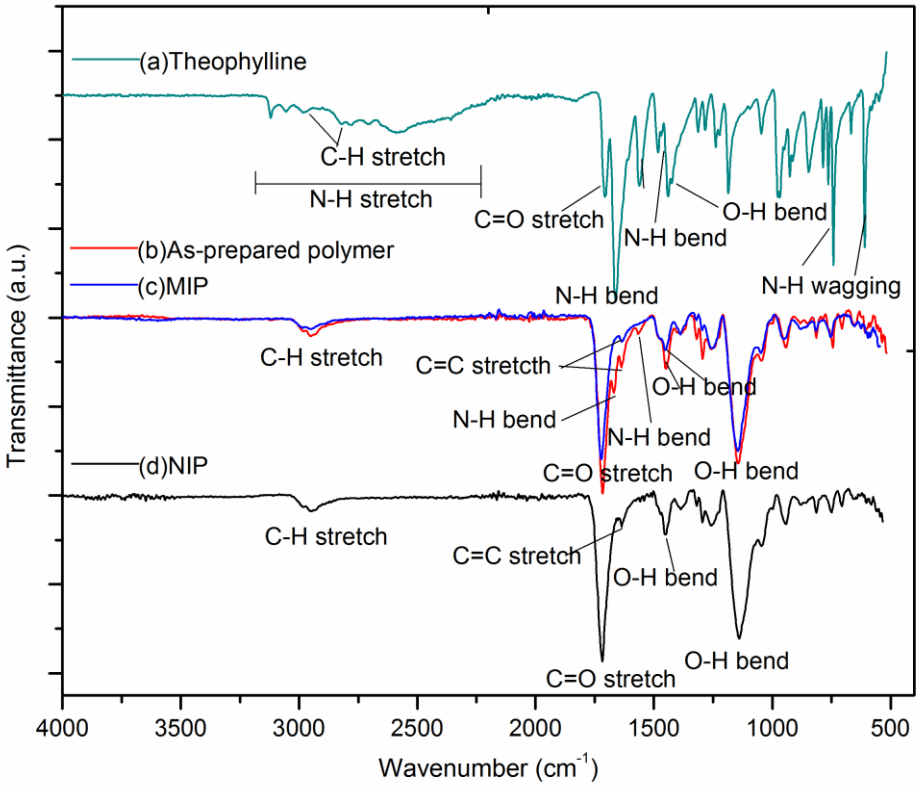

FIGURE 2. FTIR spectra of (a) theophylline, (b) as-prepared polymer, (c) MIP, and (d) NIP

3.2. Template Molecule Removal

In a molecularly imprinted polymer, the extraction of the template molecules was conducted to provide guest-binding sites in the imprinted polymer. A free-template MIP was required for optimum guest-binding process. As shown in Fig. 4, after the template extraction process, sites in the polymer matrix that was previously filled with theophylline left as cavities. Choice of solvents used in this process was quite critical, since it has to be able to extract the template without damaging the polymer. For that purpose, acetonitrile, methanol, and acetic acid were used because they were able to dissolve theophylline, but not the polymer. Methanol/acetic acid mixture with volume ratio of 9/1 was employed as desorbent solvent. The addition of 10% acetic acid has been proved to be able to increase the desorption capacity of methanol [8].

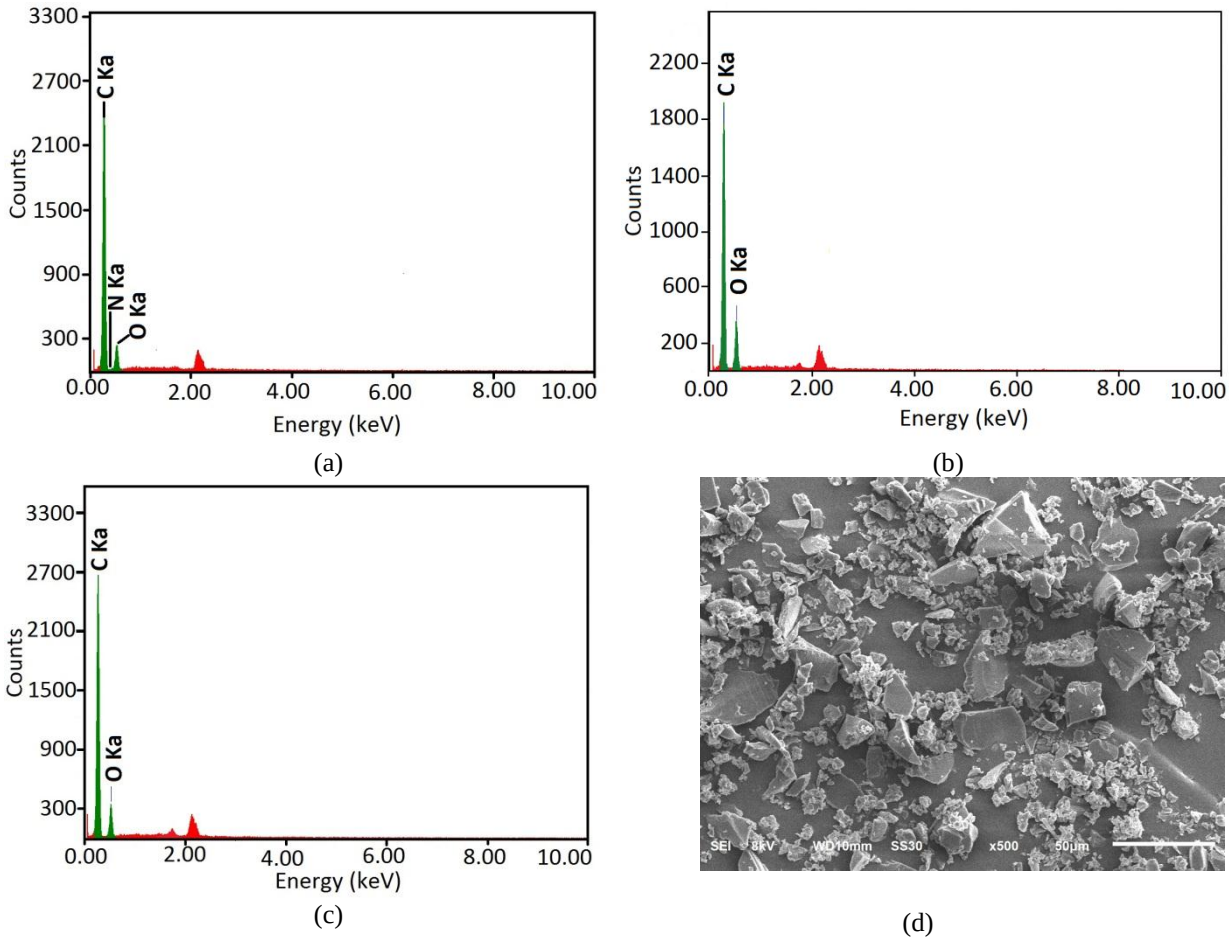

FIGURE 3. EDS spectra of (a) as-prepared polymer, (b) NIP, (c) MIP, and (d) SEM image of MIP

Compared to the FTIR spectrum of the as-prepared polymer (Fig. 2.(b)), the spectrum of MIP (Fig. 2.(c)) did not show any bands associated with theophylline. Transmission bands at 1670 and 1566 cm^{-1} associated with N-H vibration of amino group of theophylline were not observed in the spectrum. This result suggests that the template removal process was successfully carried out. The EDS spectrum (Fig. 3.(c)) also shows the same result. The MIP did not contain any N atoms, indicating a free-theophylline MIP, with mass percentage of C and O were 86.04 and 13.96%, respectively.

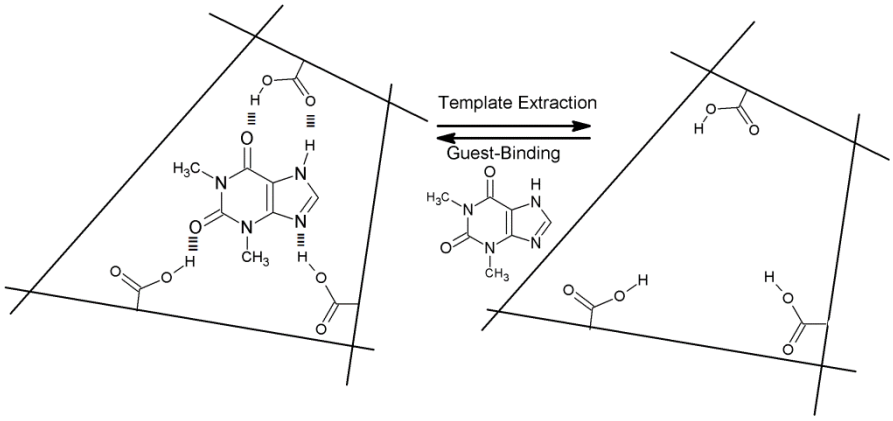

FIGURE 4. Schematic illustration of theophylline extraction from the as-prepared polymer and its guest-binding [6]

3.3. Study of Guest-Binding of the MIP

After template removal process, the MIP has specific cavities or imprint that complementary chemically and sterically to the template molecule [15]. These cavities stored the memory of shape, size, and any

physicochemical properties of the template molecules. The free-template MIP is expected to be able to recognize the template molecules or its analogues and bind the molecules, as shown in Fig. 4 for guest-binding process. The binding capacity of the MIP was studied through Batchwise guest-binding experiment. The incubation of the free-template MIP in theophylline solution for 14 h under magnetic stirring was assumed to be able to achieve an equilibrium state, that is, the MIP has reached its maximum capacity to bind theophylline.

Lata et al. [7], Khorrami and Rashdipur [8], and Mullet and Lai [16] reported that a MIP shows the best selectivity and guest-rebinding capacity when the Batchwise guest-binding experiment was conducted in the solvent used in the preparation of MIP. However, because chloroform wavelength cut-off is in the range of 240 to 260 nm, it is too close to λ_{max} of theophylline (272 nm). Therefore, by utilizing this solvent, quantitative analysis from UV/Vis spectrometer measurement is not able to be conducted because the UV absorbance of theophylline will be unstable. Besides that, the polymer could not be separated through centrifugation when chloroform was the only solvent used in the Batchwise experiment. In order to overcome this problem, chloroform/methanol mixture with volume ratio of 1/1 was used as the solvent in the Batchwise experiment. In this solvent, the UV absorbance of theophylline was stable (λ_{max} is at 272 nm). Methanol was used as the solvent mixture due to its ability to dissolve theophylline properly. Besides that, its cut-off wavelength is in the range of 205 to 240 nm, which is quite far from the λ_{max} of theophylline.

By utilizing Eq. (1), for initial concentration of theophylline in methanol/chloroform (1/1, v/v) of 0.333 mM, the amount of theophylline bound to the MIP was 23.22 μmol per gram of polymer. As a reference, the NIP was also employed in the Batchwise experiment as the guest-binding agent. Compared to the MIP, the binding capacity of the NIP was only 3.73 μmol/g of polymer. This results show that the MIP has higher binding affinity than the NIP.

4. Conclusion

A modified bulk polymerization method has been conducted in obtaining a MIP based on MAA as monomer and theophylline as template. The polymerization process was carried out at 60^{o}C for 7 h after cooling treatment of the pre-polymerization mixture. Using this method, the overall time of MIP preparation was reduced while maintaining the simplicity of the bulk polymerization method. Transmission bands associated with N-H vibration from amino group of theophylline were observed in the FTIR spectrum of the as-prepared polymer at 1670 and 1566 cm^{-1} indicating successful incorporation of theophylline in the polymer. This result was also confirmed by EDS analysis showing that N atoms of the as-prepared polymer were derived from amino group of theophylline. Furthermore, the polymer particles of the MIP were irregular in shape and size as shown by its SEM image. Study of guest-binding capacity of the MIP was carried out through Batchwise experiment. For initial concentration of theophylline in methanol/chloroform (1/1, v/v) of 0.333 mM, the amount of theophylline bound to the MIP was 23.22 μmol/g of polymer. Compared to the MIP, the binding capacity of the NIP was only 3.73 μmol/g f polymer. This result showed that the MIP has higher binding affinity than the NIP.

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