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Characterization of an Atrazine Molecularly Imprinted Polymer Prepared by a Cooling Method

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Abstract. A molecularly imprinted polymer (MIP) for atrazine was successfully prepared. Atrazine molecules as templates were incorporated into the pre-polymerization solution containing a functional monomer (methacrylic acid), a cross-linker (ethylene glycol dimethacrylate), and an initiator (benzoyl peroxide). The placement of a tube containing the pre-polymerization solution into a freezer was done to replace nitrogen pouring into the pre-polymerization solution. The sensing characteristic of the obtained MIP was examined and it was found that the amount of atrazine bound to the cavities in the MIP increases with increasing the initial concentration of atrazine. From Scatchard plots, it was found that the equilibrium dissociation constant K_D and the apparent maximum number of binding sites B_{max}, which are written as (K_D, B_{max}), are (6.4 μM, 13.41 mmol/g) and (6.5 μM, 4.55 mmol/g) for the 10 and 30 mg of MIP, respectively.

Keywords: MIP, atrazine, cavity, template, Scatchard plot.
PACS: 81.05.-t, *87.85.J-,* 81.05.Lg

INTRODUCTION

Atrazine, which is harmful toxins, is commonly used herbicides in agriculture and would have devastating effects on the environment if it is used excessively. It has been reported that the use of these herbicides can increase corn production by 6%, but a continuous the use of potentially harmful to humans and the environment [1]. Hayes also explained that atrazine may cause hormonal effects that have plagued the development of amphibious animal sex [2].

As an agricultural producing country, Indonesia has farm and estates. Certainly, the uses of pesticides (herbicides, insecticides, and fungicides) are helpful in combating the things that disturb the process plant growth. But, as a consequence, the impact of the uses of herbicides containing atrazine is extremely dangerous because it can contaminate the surrounding environment.

Molecular imprinting is a technique to produce an artificial polymer which can be applied as a chemical/biological sensor. In the technique, a functional monomer and a template are bonded by a covalent or non-covalent bond in which the functional monomer is complementary to the template. After the polymer is formed, the template molecules are removed. Consequently, the template molecules will leave holes or cavities. These cavities will remember the size, structure, and other physicochemical properties of the template. Then, the polymer is called as a molecularly imprinted polymer (MIP).

In the use of MIP as a sensor, selectivity and affinity template will increase with the concentration [3]. Until now, the MIP methods are still being developed extensively in recognizing elements in chemical and biological samples [4,5,6,7,8], medicines, and foods [10,11], because they have several advantages such as easiness in manufacturing and low cost. In the MIP, the bond strength of guest-binding sites will be determined by the value of the dissociation constant [12,13].

In the previous synthesis of MIPs, nitrogen is poured into the pre-polymerization solution to remove oxygen that disturbs the polymerization process [11]. In this paper, it will be reported a study on the replacement of nitrogen pouring into the pre-polymerization solution with the placement of a tube containing the pre-polymerization solution into a freezer. It will be shown that this cooling method can be an alternative to the previous one in making MIPs. Sensing tests of the obtained MIP for atrazine will also be demonstrated.

MATERIALS AND METHODS

Chemicals

Atrazine pestanal (molar mass of 215.68 g/mol), methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), benzoyl peroxide (BPO), methanol, acetonitrile, chloroform, acetic acid, and

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aquabidest. All the chemicals were obtained from Sigma Aldrich.

Methods

Figure 1, which is a graphical representation of making an atrazine MIP and testing its sensing characteristic, illustrates how the initial analyte (atrazine) molecules as templates are incorporated into the pre-polymerization mixture containing a functional monomer (MAA), a cross-linker (EDMA), and an initiator (BPO) for the formation of the bond between atrazine and MAA. After the polymer was formed, then by using consecutive solvents (acetonitrile, acetic acid, and aquabidest), the analyte molecules are removed so that the remaining polymer (a MIP) contains cavities which have similar shape with the analyte. When the target/guest with the same structure and properties of the template is exposed to the MIP, the MIP will recognize and be able to coordinate/bind the target/guest effectively.

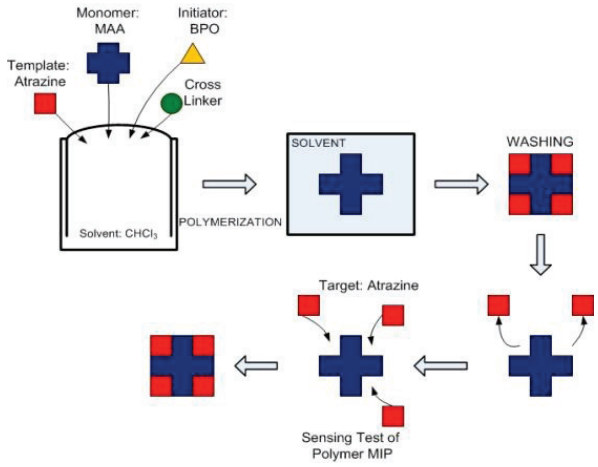

FIGURE 1. The process of making an atrazine molecularly imprinted polymer (MIP) and testing its sensing characteristic.

From the description given in Fig. 1, the steps to make an atrazine molecularly imprinted polymer (MIP) and to test a sensing characteristic of the produced atrazine are as follows.

1. Polymerization process

Into 2.1 mL of a chloroform ($CHCl_3$) solution of atrazine as a template (0.025 g) poured into each vial, MAA as a functional monomer (0.059 ml), EDMA as a cross linker (0.525 mL), benzoyl peroxide (BPO) as an initiator (0.5 g) were added. The vials, which are now as polymerization vials, were sealed and then stored in a freezer for 1 h. The polymerization vials were then placed in a water bath at 0°C and irradiated with UV light for 4 h. Finally, the vials were kept at room temperature for several days.

2. Template removal process

The obtained polymers from step 1 were crushed into particles. Then, the polymer particles were soaked in acetonitrile for 24 h and then filtered to remove the atrazine template molecules. Further template removal was achieved by successively washing the polymers in methanol/acetic acid (0.625 mL/12.5 mL), methanol/aquabidest (6.375 mL/12.5 mL), and methanol for 48 h. The template-free polymer particles were finally collected and dried under vacuum.

3. Sensing test

Firstly, atrazine standard solution of 5000 ppm as stock solution was prepared by weighing atrazine (50 mg) and dissolving it in 10 ml of methanol. A series of diluted stock solution was made by diluting 0.5, 1, 1.5, and 2 mL of the stock solution with 5 mL of methanol/acetic acid with the ratio of 1:1. The series are 500, 1000, 1500, and 2000 ppm.

Next, the MIP particles obtained from step 2 with certain mass (m) were put in 4 sample tubes and 1 mL of the diluted stock solution was added into the sample tubes. To obtain the initial consentrations of atrazine, 5 mL of chloroform, 5 mL of acetic acid, and 10 mL of methanol were added into these mixtures. Thus, a series of 0.110, 0.221, 0.331, and 0.442 mM initial concentrations were obtained. The sample tubes then stirred in for 12 h., put in a centrifuge for 15 min. to remove excess polymer, and filtered with 45-micron filter papers to collect the remaining atrazine solution.

Subsequently, the concentration of free atrazine in the remaining solution, which is called as R_{remain}, was determined by a reversed-phase HPLC. The difference of C and R_{remain} is therefore the number of guests bound to the MIP particles, which is called as B_{bound}. For all measurements, the injection volume was 50 µL, the mobile phase was 0.01 M of acetic acid in acetonitrile (50:50), the flow rate was 1 mL/min and the detection employed UV light at 260 nm.

RESULTS AND DISCUSSION

Two different quantities (10 and 30 mg) of the obtained MIP particles were used to test the sensing characteristic of the obtained MIP particles. Table 1 shows the measurement variable that is 1 mL of atrazine solutions with known initial concentrations along with the measured free atrazine concentration and the obtained amount of bound atrazine.

TABEL 1. Initial concentrations of atrazine along with their measured free atrazine concentrations and atrazine bound to the MIP particles.

MIP (mg)	Initial concentration of atrazine, C(mM)	[Atrazine] free (mM)	B_{bound} (mmol/g)	B_{bound}/C (cm^3/g)
10	0.110	0.0024	0.2267	2054
	0.221	0.0072	0.4485	2031
	0.331	0.0183	0.6571	1984
	0.442	0.0297	0.8650	1959
30	0.110	0.0020	0.0759	688
	0.221	0.0075	0.1493	676
	0.331	0.0160	0.2207	666
	0.442	0.0287	0.2890	654

Figure 2 presents saturation binding or binding isotherm curves of atrazine in which the horizontal axis represents the initial concentration of atrazine and the vertical one is the concentration of atrazine bound in the cavities of the obtained MIP particles. It is shown that the amount of atrazine bound to the cavities in the MIP particles (10 and 30 mg) increases with increasing the initial concentration of atrazine. This implies that the obtained MIP is able to sense atrazine.

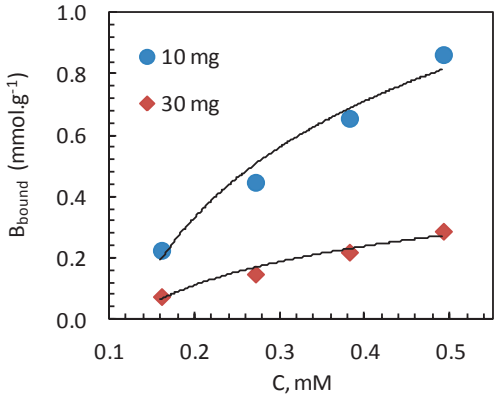

FIGURE 2. Binding isotherm or saturation binding curves for 1 mL of the atrazine with known concentrations in10 and 30 mg of the MIP particles.

To determine the apparent maximum number of binding sites and the equilibrium binding constant, the curves in Fig. 2 is re-plotted to be a Scatchard plot as given in Eq.(1).

$$\frac{B_{bound}}{C} = -\frac{1}{K_D} B_{bound} + \frac{B_{\max}}{K_D} \qquad (1)$$

where B_{bound} is the concentration of atrazine bound to the polymer, C is the guest concentration of atrazine, B_{max} is the apparent maximum number of binding sites, and K_D is the equilibrium dissociation constant.

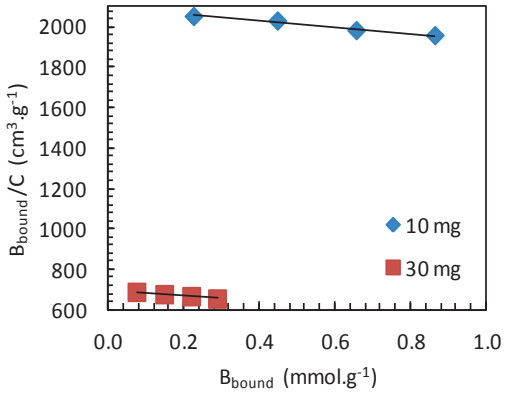

FIGURE 3. Scatchard plots for 1 mL of the atrazine solution in 10 and 30 mg of the MIP particles.

From data in Tabel 1, Scatchard plots for 1 mL of atrazine solution are made as given in Figure 3. It is found that K_D and B_{max} values, which are written as (K_D, B_{max}), are (6.4 µM, 13.41 mmol/g) and (6.5 µM, 4.55 mmol/g) for 10 and 30 mg of the MIP particles, respectively. The apparent maximum number of binding sites B_{max} for the 30 mg of MIP particles is 4.55 mmol/g, which is only about 1/3 of that for the 10 mg of MIP particles. It means the absolute apparent maximum number of binding sites is the same (about 13.65 mmol).

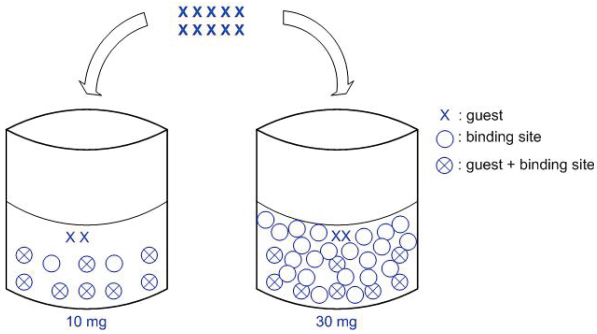

FIGURE 4. Illustration of the binding process of atrazine in the 10 and 30 mg of MIP particles.

A possible explanation is given by an illustration in Figure 4. Compared to the number of available cavities (binding sites) in the 10 mg of MIP particles, that in the 30 mg of MIP particles is three times more. Therefore, the available binding site that can be utilized in the 30 mg of MIP particles is about

1/3 of that in the 10 mg of MIP particles for initial concentrations of atrazine (guest) below a limit value in which all of the guests are bound to the binding sites in the 10 mg of MIP particles.

CONCLUSION

The synthesis of an atrazine molecularly imprinted polymer (MIP) has been successfully carried out by placing a tube containing the pre-polymerization solution of methacrylic acid, ethylene glycol dimethacrylate, and benzoyl peroxide into a freezer. From sensing test of the obtained MIP, it has been found that the amount of atrazine bound to the cavities in the MIP increases with increasing the initial concentration of atrazine. Moreover, it has been found from Scatchard plots that the equilibrium dissociation constant K_D and the apparent maximum number of binding sites B_{max}, which are written as (K_D, B_{max}), are (6.4 μM, 13.41 mmol/g) and (6.5 μM, 4.55 mmol/g) for the 10 and 30 mg of MIP, respectively.

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