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MIP-TFMAA-co-TRIM as Selective Adsorption of β-sitosterol

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ABSTRACT

A functional monomer of trifluoromethylacrylic acid (TFMAA), a cross-linker of trimethylolpropane trimethacrylate (TRIM) and a template molecule of β-sitosterol have been used to produce a molecularly imprinted polymer, MIP_TFMAA-co-TRIM by a polymerization process based on non-covalent interaction between the monomer and the template. After the removal of the template, we used the product of MIP as an adsorbent of β-sitosterol and determined the adsorption capacity. The adsorption-desorption process for β-sitosterol and the selectivity test for β-sitosterol and cholesterol were also studied. High Performance Liquid Chromatography (HPLC) and UV-vis spectrophotometry were methods for measuring the amount of .materials adsorbed. Langmuir and Freundlich equations were models used to study the adsorption isotherm of the removal of β-sitosterol. The adsorption of β-sitosterol on MIP_TFMAA-co-TRIM followed the Freundlich isotherm with a capacity of 0.61 mg/g. The first and the second adsorption-desorption processes showed that the recovery percentages of β-sitosterol from MIP_TFMAA-co-TRIM were 66.36 and 40.56%, respectively. The MIP was more selective for β-sitosterol than for cholesterol.

Keywords: Adsorption capacity; MIP; TFMAA; TRIM, selectivity

1. INTRODUCTION

It is well known that interactions between monomers and template molecules through covalent or non-covalent interactions in a polymerization process will produce molecularly imprinted polymers (MIPs). In the synthesis of MIP, the non-covalent interaction is preferred because of the simplicity and flexibility of the process [1]. Hydrogen and ionic bonds, as well as hydrophobic and dipole-dipole interactions between the template molecule and the monomer, are the possible interactions that occurred in the formation of MIP [2,3]. It is easier in the non-covalent interaction to remove the template by extraction with an organic solvent than in the covalent interaction [3]. The removal of the template from the polymer produces, cavities with the same shape and size as the template. [3,4]. The cavities of the MIP have specific functional groups to the target molecule [5,6]. As a consequence, the MIP has high selectivity to adsorb the target molecule [7]. Because of the fact, the problem faced in separating compounds having similar structures, such as cholesterol, ergosterol, stigmasterol, and β-

sitosterol can be solved [8]. Therefore, the material can be utilized in isolating and purifying compounds in complicated natural products.

It was reported that sterol compounds have mostly been found in plants [8]. One dominant compound in plants is β-sitosterol [9]. The structure and physical properties of the compounds are similar to that of other sterol compounds. Therefore, it is difficult to separate the material from a mixture [10]. Based on this fact, β-sitosterol can be used as the template (target molecule) in the synthesis of a MIP. It is useful to selectively adsorb the target molecule that usually interferes in the isolation process of compounds in plants.

In this study, a MIP was prepared using TFMAA, TRIM, and β-sitosterol as a functional monomer, a cross-linker, and a template molecule, respectively to form MIP_TFMAA-co-TRIM. We used the MIP to adsorb the target molecule and to study the adsorption-desorption processes. The selectivity of this MIP was studied by comparing the adsorption of the target molecule and that of cholesterol.

2. METHODOLOGY

A. Materials and Apparatus

Materials used were 97% β-sitosterol (Sigma-Aldrich), trifluoromethylacrylic acid 98 % (TFMAA) (Sigma-Aldrich), ethylene glycol dimethacrylate (EGDMA) (Sigma-Aldrich), trimethylolpropane trimethacrylate (TRIM) (Sigma-Aldrich), 2,2'–azobisisobutyronitrile (AIBN), cholesterol 99% (Sigma-Aldrich), toluene, methanol (HPLC Grade), tetrahydrofuran (THF) p.a., acetic acid p.a., and pure nitrogen gas from Merck. All reagents were used without any treatment.

In this study, we used the apparatus as follows: glassware, analytical Balance (Ohaus), oven, water bath (Thermo Electron, 200748), sonicator (Elma, S40H), shaker, mini glass column (diameter and length = 3 cm), micro-pipettes of 10, 100, 1000 µL (Eppendorf), Solid Phase Extraction tool, mini peristaltic pump (Gilson), pH meter (Hanna), High Pressure Liquid Chromatography (HPLC) of Agilent 1260 infinity with column type of Cronus RP E18 C and column size of 12.5 x 0,4 cm, Thermogravimetric Analyzer (TGA) NETZSCH STA 449F1, Ultra Violet-Visible (UV-Vis) Spectrophotometer of Agilent 8453 and Shimadzu.

B. Methods

B.1 Synthesis of MIPs

The MIP_TFMAA-co-TRIM samples were synthesized using the procedure described in the previous study [11]. The MIP material was different from the NIP material showed in the FTIR spectra and the images of SEM [11]. SEM EDX and thermogravimetric analysis were used in this research.

B.2. Determination of adsorption capacity

The following procedure was conducted to determine the amount of β-sitosterol adsorbed on MIP. Solutions of β-sitosterol (3 mL) in various concentrations (2,

4, 6, 8, and 10 ppm) were prepared at the optimum pH of 6, the pH obtained from the previous work [11]. The solutions were inserted into vials containing 20 mg of MIP and mixed by shaking at 30 ^{0}C (room temperature) for 60 min (the optimum contact time that previously determined) [11]. The MIP sample was separated from the solution by filtering, and the amount of β-sitosterol in the filtrate was determined by a UV spectrophofometer at a wavelength of 202 nm.

The amount of β-sitosterol adsorbed by MIP_TFMAA-co-TRIM was determined by Eq. 1 as described in [12],

$$q_e = \frac{(C_o - C_e)\,V}{m} \quad (1)$$

where q_e is the amount of β-sitosterol adsorbed per gram of adsorbent (mg/g), C_o is the initial concentration (mg/L), C_e is the final concentrations (mg/L), V (L) is the solution volume and m is the weight of adsorbent in gram [12]. The adsorption isotherms were studied using Langmuir and Freundlich models.

B.3. MIP_TFMAA-co-TRIM selectivity test

A mini-column containing 50 mg of MIP saturated previously with methanol was used to study the selectivity of MIP_TFMAA-co-TRIM. A 10 ppm β-sitosterol standard solution (0.5 mL) was mixed with a standard solution of cholesterol with a concentration of 10 ppm (0.5 mL). A carrier solvent (2 mL) with two different composition ratios of methanol and water (90:10, and 100:0) were added. The mixture of solvents was passed to a mini-column containing the MIP, and 1 mL of the solvent coming out from the column was collected in a vial. The trapped β-sitosterol and cholesterol were removed from the MIP by passing 100% methanol through the column and 1 mL of the solvent coming out from the column was also collected in a vial. Finally, 1 mL of each eluate was analyzed using HPLC. The adsorption of β-sitosterol and cholesterol was then determined.

B.4. Adsorption Desorption Test of β-sitosterol

A mini-column containing 50 mg of MIP saturated with methanol was prepared to be used in adsorption-desorption tests. A standard solution of 10 ppm β-sitosterol (0.5 mL) was mixed with 2 mL of the carrier solvent, *i.e.* the solvent containing methanol and water with the best composition ratio used in the selectivity test. The mixture was then passed through the column containing 50 mg of MIP to be adsorbed. Furthermore, the solvent was run through the column by using a peristaltic pump. The eluate (1 mL) was collected. After that, the desorption of β-sitosterol adsorbed on the MIP was conducted using pure methanol (100%). Finally, the eluate collected was analyzed using a UV spectrophotometer.

3. RESULTS AND DISCUSSION

A. Characterization of MIP with SEM-EDX and TGA

A previous study [11] found that by using SEM images, MIP_TFMAA-co-TRIM had more porous surface compared to NIP_TFMAA-co-TRIM. The results were supported by SEM-EDX data given in Table 1.

The atomic percentage of carbon in the MIP sample before extraction (MIP_TFMAA-co-TRIM$_{BE}$) is higher than that in the MIP sample after extraction (MIP_TFMAA-co-TRIM$_{AE}$). This showed the existence of β-sitosterol in the sample before extraction.

Table 1. Summary of SEM-EDS analysis of NIP_TFMAA-co-TRIM, MIP_TFMAA-co-TRIM$_{BE,}$ and MIP_TFMAA-co-TRIM$_{AE}$

Element	Weight %			Atomic %		
	NIP	MIP$_{BE}$	MIP$_{AE}$	NIP	MIP$_{BE}$	MIP$_{AE}$
C	77.97	75.59	74.81	82.79	81.21	80.54
O	19.25	17.41	18.15	14.04	14.04	14.67
F	2.78	7.00	7.04	4.75	4.75	4.79

It is clear that the percentage of carbon (C) decreases, whereas that of oxygen (O) increases after the removal of the template. The amount of β-sitosterol released from the polymer is small showed by the small decrease in the former and the small increase in the later. This is in parallel with the SEM images reported in the previous study [11] in which there were not many pores shown in the MIP_TFMAA-co-TRIM surface. This fact influenced the ability of MIP_TFMAA-co-TRIM in binding the target molecule. The atomic percentage of fluorine (F) in MIP_TFMAA-co-TRIM$_{BE}$ is almost the same as in MIP_TFMAA-co-TRIM$_{AE}$, indicating that the polymer matrix was not damaged during the extraction.

Thermograms of MIP and NIP materials are given in Fig. 1. It is obvious that the termogram of NIP_TFMAA-co-TRIM has a similar pattern to that of MIP_TFMAA-co-TRIM$_{AE}$. The result was obtained because β-sitosterol has been removed from the MIP after extraction , meaning that both samples did not contain β-sitosterol. The TGA and DTG heating curves are given in Fig.2.

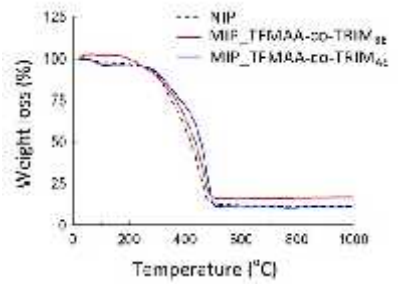

Fig. 1. Thermograms of NIP_TFMAA-co-TRIM and MIP_TFMAA-co-TRIM

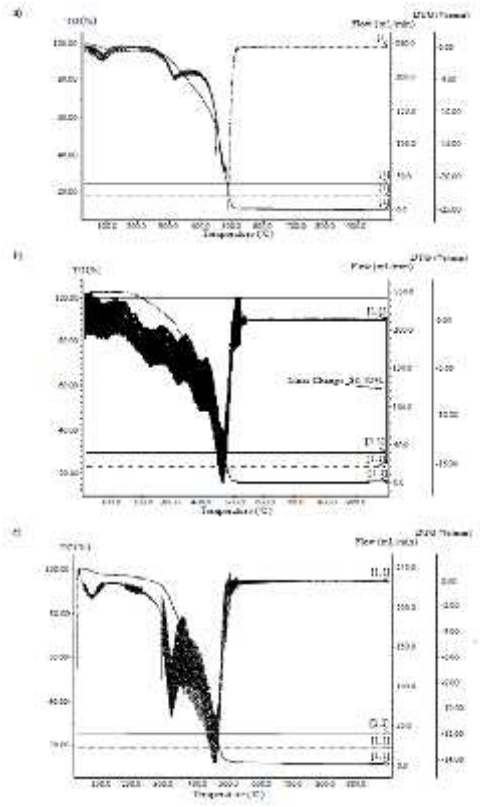

Fig. 2. Differential Thermograms of (a) NIP_TFMAA-co-TRIM, (b) TFMAA-co-$TRIM_{BE}$, and (c) MIP_TFMAA-co-$TRIM_{AE}$

NIP_TFMAA-co-TRIM, IP_TFMAA-co-$TRIM_{BE}$, and MIP_TFMAA-co-$TRIM_{AE}$ begin to loss their masses from a temperature of 37.54 to 237.5ºC, 32.81 to 175.9 ºC, and 30.9 to 202.9 ºC, respectively. At those temperature, water molecules were released from polymers during the heating process. The peaks at 317.5ºC for the NIP_TFMAA-co-TRIM, 318.9 ºC for MIP_TFMAA-co-$TRIM_{BE}$, and 315.9 ºC for MIP_TFMAA-co-$TRIM_{AE}$ appear due to the loss of fluorine by heating.

B. The effect of initial concentration of β-sitosterol

With increasing the initial concentration used, the adsorption of β-sitosterol in the MIP increases as clearly shown in Fig. 3.

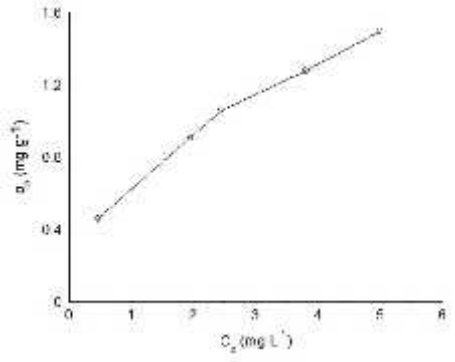

Fig. 3. The effect of the initial concentration of β-sitosterol to the amount of β-sitosterol adsorbed by MIP_TFMAA-co-TRIM (pH = 6, contact time = 60 min)

The amount adsorbed remains increase at the measured concentration. Therefore, Langmuir and Freundlich models were used to determine the adsorption capacity.

The Langmuir isotherm curve was obtained by plotting $1/q_e$ Vs $1/C_e$ as can be seen in Fig. 4a. Using the Langmuir equation, the adsorption capacity can be deduced [13, 14]. The linear form of the equation is illustrated in Eq. 2,

$$\frac{1}{q_e} = \frac{1}{Q_o b}\left(\frac{1}{C_e}\right) + \frac{1}{Q_o} \qquad (2)$$

where Q_o is the adsorption capacity, q_e is the amount of β-sitosterol adsorbed (mg/g), b is connected with the adsorption energy. Fig. 4b shows the Freundlich isotherm produced by plotting log (x/m) vs log C_e. The adsorption capacity can be obtained from the Freundlich equation [14, 15] as given in Eq. 3,

$$\log \frac{x}{m} = \log k_f + \frac{1}{n} \log C_e \qquad (3)$$

where C_e is the equilibrium concentration of β-sitosterol in solution (mg/L), x is the amount of β-sitosterol adsorbed (mg), and m is a mass of MIP (g). K_f and n are constants related to factors affecting the adsorption capacity and adsorption intensity.

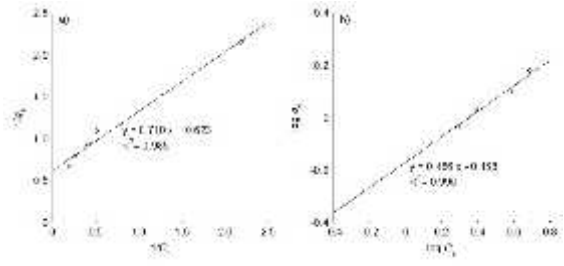

Fig. 4 Adsorption isotherms of a) Langmuir and b) Freundlich for the adsorption of β-sitosterol in MIP_TFMAA-co-TRIM.

Fig. 4 shows that the correlation coefficient (R^2) for the Freundlich isotherm is

slightly higher than that for Langmuir isotherm. This indicates that the Freundlich model better fits the adsorption of β-sitosterol by MIP_TFMAA-co-TRIM with an adsorption capacity of 0.67 mg/g. The similar results were reported from the removal of 2,4-dichlorophenol by a MIP methacrylic acid functionalized β-cyclodextrin using 2,4-dichlorophenol as the template [15] and the adsorption of melamine by melamine-MIP-9-vinylcarbazole [16]. The two studies showed that the adsorptions followed the Freundlich model. Our previous studies using MIP_TFMAA-co-EDGMA [17] and MIP_MAA-co-EDGMA [18] as adsorbent of β-sitosterol also obeyed the Freundlich model.

The comparison of adsorption capacity of β-sitosterol using the MIP sample used in this study to the MIP samples used previously is given in Table 2.

Table 2. The adsorption capacity of MIPs to β-sitosterol

MIP	Adsorption capacity, k (mg/g)*	Ref.
MIP_TFMAA-co-EDGMA	0.49	[17]
MIP_MAA-co-EGDMA	1.05	[18]
MIP_TFMAA-co-TRIM	0.67	**

* Calculated based on Freundlich isotherm
** Current study
EDGMA = ethylene glycol dimethacrylate
MAA = methacrylate acid

The adsorption capacity of MIP_TFMAA-co-TRIM is higher than that of MIP_TFMAA-co-EDGMA but lower than that of MIP_MAA-co-EDGMA. The reason for the former fact is that the cross-linker of TRIM has more vinyl groups than the cross-linker of EDGMA has. The vinyl groups have a contribution to the binding with the monomer that can produce strong and stable polymers [1].

The reason for the later fact is that the monomer of TFMAA contains F atom that is very electronegative. This can affect the interaction of the monomer with the cross-linker (TRIM). As a consequence, the formation of the polymer will not be maximized. According to Beltran *et al.* [19], the stability of MIP is very dependent on the cross-linker as well as the monomer and the adsorption capacity of MIP to the target molecule is mostly affected by the stability.

C. Adsorption-desorption of β-sitosterol

It is necessary to perform the adsorption-desorption test to find out the possibility of the adsorbent to be reused. The test was studied to evaluate the percentage of β-sitosterol recovered from MIP_TFMAA-co-TRIM. The target molecule that can be recovered from MIP_TFMAA-co-TRIM in the first adsorption-desorption test was 66.36%, whereas the recovery percentage of the target molecule in the second adsorption-desorption process was 40.56 %. This means that the polymer can only be used once because after the second adsorption, the percentage of β-sitosterol that can be recovered was less than 50%. When we compared the result obtained in this study to the ones previously studied using MIP_TFMAA-co-EDGMA [17] and MIP-MAA-co-TRIM [18], the results obtained

were different. The recovery percentage of β-sitosterol in the second adsorption-desorption process was in the order as follows: MIP_MAA-co-EDGMA > MIP_TFMAA-co-TRIM > MIP_TFMAA-co-EDGMA. This shows that the stability of the MIP sample produced using MMA as a monomer is higher than that produced using TFMAA. In addition, the stability of MIP is also affcted by the cross-linker. As a cross-linker, TRIM can produce MIP that more stable compared to EDGMA.

D. Selectivity of MIP

It is required in this study to get information of the best composition of solvent used to carry the target compound. The solvent has to bind more β-sitosterol than the comparison compound having a similar structure. In this case, cholesterol was used as the comparison compound. The adsorption depends on the solvent polarity and the type of MIP used. When we used methanol without water, the adsorption effectivity of β-sitosterol was 100%, whereas that of cholesterol was 58.44%. The best composition was obtained in the solution of methanol-water with a ratio of 90:10. Using the solution, the adsorption effectivity of MIP to β-sitosterol was 100%, whereas that to cholesterol was 19.29%. This indicates that the selectivity of the MIP to bind β-sitosterol is considerably higher than to cholesterol.

4. CONCLUSION

MIP_TFMAA-co-TRIM has been successfully synthesized through a non-covalent interaction in the polymerization process. The MIP produced can be used as a selective adsorbent for β-sitosterol. The adsorption fitted the Freundlich model with an adsorption capacity of 0.67 mg/g. The recovery of β-sitosterol after the first and the second adsorption-desorption processes was 66.36 and 40.56%, respectively. The MIP has considerably higher selectivity to β-sitosterol than to cholesterol.

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