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Synthesis, Characterization, And Optimization Of β-Sitosterol Imprinted Polymers Using TFMAA As Functional Monomer

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ABSTRACT

Synthesis of Molecularly Imprinted Polymers (MIP) has been conducted using trifluoromethylacrylic acid (TFMAA) as a functional monomer, trimethylolpropane trimethacrylate (TRIM) as a cross-linker, 2,2‘-azobis isobutironitril (AIBN) as an initiator, and toluene as porogen solvent, and β-sitosterol as template molecule. The MIP was synthesized using imprinting molecular technique through polymerization process. The template molecule was used as a porous template on polymer to form MIP. The polymer could be applied as the high affinity adsorbent for β-sitosterol. Therefore, β-sitosterol compound as a template molecule was selected to print the cavities in the polymer. Functional groups and the morphology of MIP were determined by using Fourier Transform Infrared (FTIR) spectrophotometer and Scanning Electron Microscopy (SEM), respectively. The ability to adsorb β-sitosterol was optimized with variables of contact time and pH using high performance liquid chromatography (HPLC). Functional groups involved in the formation of MIP were O-H, C=C, C=O and C-F. The surface morphology of MIP was coarser and more porous than that of NIP. The adsorption of β-sitosterol in MIP achieve the optimum at a pH of 6 and a time of 60 minutes. The amount of β-sitosterol adsorbed in MIP was higher than that in NIP. The adsorption β-sitosterol in MIP followed the pseudo second order kinetic model with a rate constant (k_2) of 1.7895 g min^{-1} mg^{-1}.

Key words: Molecularly imprinted polymers, functional monomer, cross linker, optimization, and β-sitosterol

Introduction

Selective extraction of a compound in a sample can be conducted with Solid Phase Extraction (SPE), but the method has limitation, especially if a traditional adsorbent is used in the process of extracting a compound in samples with complex matrices[12]. Some compounds other than the targeted compound will also be extracted[2]. Traditional adsorbents of SPE have low selectivity and adsorption capacity[12]. Therefore, a new material is required to traditional adsorbent in the extraction process. Modification of adsorbent is important to be done in order to improve the adsorbent selectivity. A molecular imprinting technique[9] is one alternative way to produce a new material with promising selective molecular recognition ability. Molecular imprinting technique requires a template molecule to make cavities in the appropriate polymer after removal of the template[4].

A synthetic polymer is made from the functional monomer and a template molecule via polymerization with the aid of a cross linking agent cross linkers[3,5]. Functional monomers plays an important role in the synthesis of MIP, because–the use of the proper functional monomer will produce appropriate interaction with the template molecule[2] Trifluoromethyl acrylic acid (TFMAA) is an acidic functional monomer acidic commonly used in the synthesis of MIP because of its hydrogen bond strength and its ability as a hydrogen donor[6]. Although the functional monomer used has proven its superiority, a cross linker is also very important because it can affect the morphology of the polymer matrix, stabilize the sides of the binding, and maintain the mechanical stability of the polymer matrix[2]. TRIM is made of a combination of three functional monomers, therefore it is superior in some applications because of its high density[7,10]. Zhu, et. al. (2007), reported that the use of the combination of TFMAA as a functional monomer, TRIM as cross-linker and 17-β-estradiol as a template molecule in the polymer synthesis produced a polymer having a stable size, high affinity and selectivity to 17-β-estradiol as a target molecule. Generally, MIP uses organic molecules as molecular templates and cavities formed after the removal of templates in the MIP will have active sites to target molecules[1].

In this study, a MIP will be synthesized using TFMAA monomers, a cross linker of TRIM and a template of β-sitosterol to obtain MIP_TFMAA-co-TRIM having high affinity and selectivity to β-sitosterol. The template is generally found in plant or biological samples and is usually difficult to be separated from other compounds[14]. Therefore, the success of the MIP to selectively recognize the target molecule will be useful for separation of β-sitosterol in an isolation process of natural products. The resulted MIP_TFMAA-co-TRIM is characterized by FTIR and SEM. The MIP adsorption ability against β-sitosterol is determined by the effect of pH and time.

Materials and Methods

Material : Trifluoromethylacrylic acid (Sigma-Aldrich) as a functional monomer, β-sitosterol (Sigma-Aldrich) as a template molecule, 2,2'-azobis isobutironitril (Sigma-Aldrich) as initiator and TRIM (Sigma-Aldrich) as *cross linker*. Grade HPLC Methanol (Merck). Equipment used included glassware, digital balance, oven, shaker, water bath, HPLC (Shimadzhu : column C18, Shimpack VP ODS, 250 x 4,6 mmID), FTIR (Shimadzhu : IRPrestige-21), and SEM (Tescan Vega 3SB).

Eksperiment : Synthesis MIP_TFMAA-co-TRIM. The TFMAA functional monomer as many as 2 mL (14,2 mmol) was dissolved in a round bottom flask containing 10 mL (9.41 mmol) of toluene as a porogen solvent. The solution was then mixed with 0.05 g (0,12 mmol) of a template molecule (β-sitosterol), 3 mL (9.3 mmol) of TRIM and 0.05 g (0,3 mmol) of AIBN. A nitrogen gas was flowed into the solution to remove oxygen. Furthermore, the polymerization process was carried out in a water bath at 55°C for 24 hours. A polymer produced from the polymerization process was dried for 48 hours, washed with tetrahydrofuran, methanol: acetate acid (9:1), and aquabidest to remove the template, porogen solvents and other compounds. The polymers was washed and dried at 50°C. The dried polymer was MIP and ready to be used as a selective adsorbent. Non Imprinted Polymers (NIP) was prepared without a template molecule with the same synthesis procedures as MIP.

Functional groups involved in the formation of MIP_TFMAA-co-TRIM were analysed using Fourier Transform Infrared (FTIR). The morphology of MIP_TFMAA-co-TRIM was characterized using (SEM)

The MIP and NIP (20 mg) that have been synthesized were separated put into 2 Erlenmeyers, subsequently added with 3 mL of β-sitosterol standard solution with a concentration of 10 ppm. The solution was stirred with a shaker for one hour at room temperature and filtered. The concentrations of β-sitosterol was analysed by HPLC. The β-sitosterol amount adsorbed (mg) per gram of adsorbent (MIP) was determined by equation (1)

$$q_e = \frac{(C_0 - C_e)\, V\,(L)}{m\,(g)} \qquad (1)$$

Where q_e is the amount of β-sitosterol adsorbed per weight of adsorbent (mg/g), C_o and C_e are the initial and equilibrium concentrations (mg/L), respectively, V (L) is the volume of solution and m (g) is the weight of adsorbent[11,12,13].

The pHs used to determine the optimum pH were 4, 5, 6, 7. MIP (20 mg) synthetized was put into four erlenmeyers, and 3 mL of 10 ppm β-sitosterol was added into the Erlenmeyer. The pH of the solution was adjusted to the certain pH. The solution was then stirred with a shaker at room temperature for 12 hours. The β-sitosterol concentrations were determined by HPLC. The optimum pH is the pH where the greatest concentration of β-sitosterol adsorbed.

Four Erlenmeyers were filled with 20 mg MIP synthesized, and 3 mL of 10 ppm β-sitosterol solution was added with a pH of 6 into the solution. Kinetic adsorption was determined by setting the experiment at different times, stirred with a shaker for 30, 60, 90, 120 min at room temperature. The concentrations of β-sitosterol were analysed by HPLC.

The optimum time is a time used at the highest concentration of β-sitosterol adsorbed. The adsorption kinetics was determined by using the first and pseudo second orders.

The equations are presented in equations (2) and (3), respectively,

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \qquad (2)$$

Where k_1 (min^{-1}) is the rate constant on the pseudo second order, q_e and q_t show the number of target molecules adsorbed (mg/g) at equilibrium and at a certain time, t (minutes). Then, the equation integrated and applied conditions $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$,

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (3)$$

Where k_2 is the rate constant on the pseudo second order ($\text{g.mg}^{-1}\ \text{min}^{-1}$).

Plots t/q_t vs t will provide a straight-line curve, if the adsorption follows the pseudo second-order kinetics and the values of q_e and k_2 can be calculated from the slope and intercept[8,13].

Results and Discussion

Molecularly Imprinted Polymers (MIP_TFMAA-co-TRIM) and Non Imprinted Polymers (NIP_TFMAA-co-TRIM) synthesized were white powders

FTIR data explain functional groups that involve in the formation of polymer. Results of functional groups characterization on MIP_TFMAA-co-TRIM and NIP_TFMAA –co-TRIM can be seen in Figure 2.

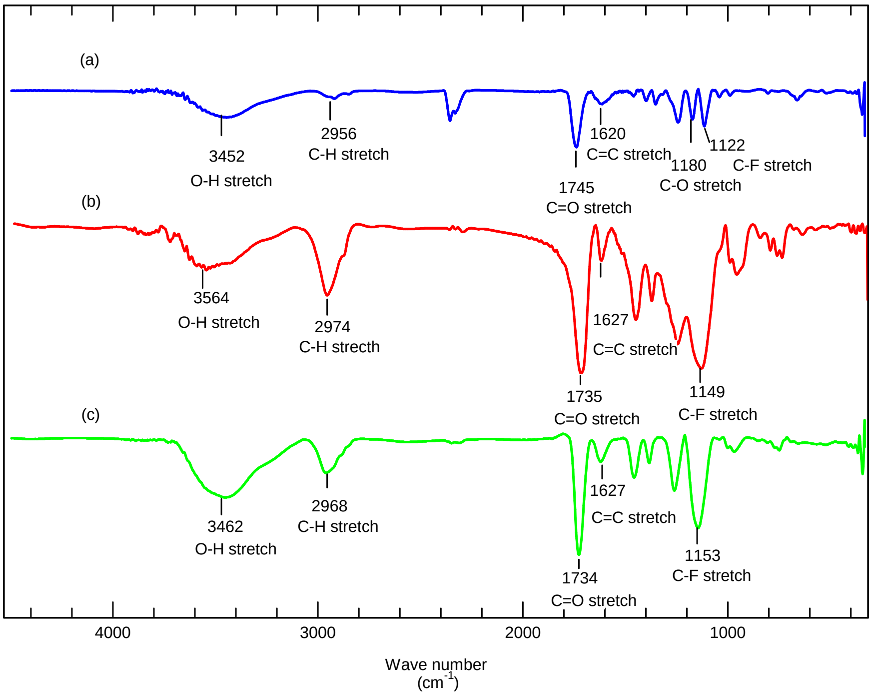


Figure 2 FTIR Spectra of a) functional monomer of TFMAA, b) NIP_TFMAA-co-TRIM, and c) MIP_TFMAA-co-TRIM

Results of FTIR analysis for NIP_TFMAA-co-TRIM and MIP_ TFMAA-co-TRIM indicate a change in the absorption vibrations compared to the functional monomer used. A Strong absorption peak of C-F at TFMAA appears at a wave number of 1122 cm^{-1}. However, after polymerization reaction, the vibration is no longer observed at the spectra of NIP_TFMAA-co-TRIM and MIP_TFMAA-co-TRIM. This indicates that there has been interaction between the C-F groups of TFMAA with other functional groups. The absorption vibration of C=C on TFMAA is observed at a wave number of 1620 cm^{-1} The peak still appears in the spectra of NIP and MIP. The absorption intensity of the peak after the polymerization. This suggests that the formation of radicals from the group C=C in the monomer TFMAA in presence of an initiator was not optimal, because C-F group in TFMAA as functional monomer which Flour (F) is highly electronegative. An absorption peak of O-H in TFMAA is at 3452 cm^{-1}, but after the formation of NIP_TFMAA-co-EGDMA and MIP_TFMAA-co-EGDMA the peak shifts to wave numbers of 3564 cm^{-1} and 3462 cm^{-1}, respectively. This happens because the functional groups of O-H undergo interaction with other functional groups to form hydrogen bonds. Functional group C=O of TFMAA also undergoing a shift wavenumber from 1180 cm^{-1} to 1149 cm^{-1} and 1153 cm^{-1} for NIP_TFMAA-co-TRIM and for MIP_TFMAA-co-TRIM, respectively. The condition was affected by functional group C=O that also undergoing interactions with other functional groups like functional groups O-H or C-F.

The morphology of NIP_TFMAA and MIP_TFMAA was characterized using SEM. Figure 3 shows the images of the polymers.

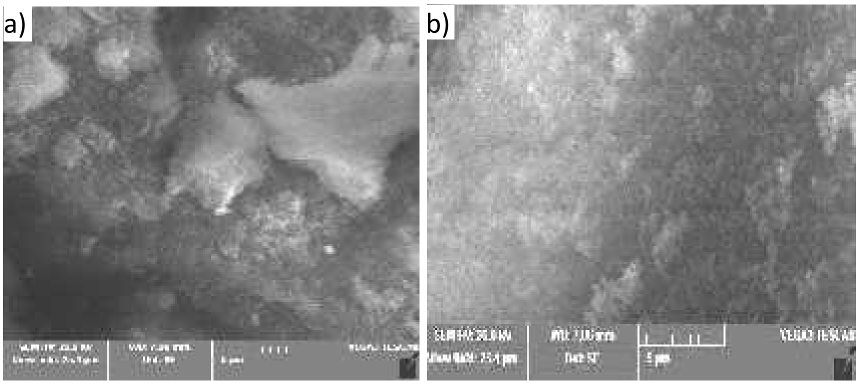


Figure 3 SEM images at a magnification of 5000x of a) NIP_TFMAA-co-TRIM and b) MIP_TFMAA-co-TRIM

As seen in Figure 3, the surface of MIP_TFMAA-co-TRIM is more porous and rougher than that of NIP_TFMAA-co-TRIM. The porousness and roughness of MIP should be considered as the formation of the empty molecular cavities after the removal of β-sitosterol.

Adsorption studies were conducted to determine the ability of NIP_TFMAA-co-TRIM and TRIM MIP_TFMAA-co-TRIM to adsorb (rebind) the target compound. The concentration of β-sitosterol in the adsorption process by MIP was determined by using HPLC. Adsorption ability of NIP_MAA and MIP_MAA can be seen in Table 1

Table 1 The amount of -sitosterol adsorbed by NIP_TFMAA-co-TRIM and MIP_TFMAA-co-TRIM

Polymer kind	Amount of β-sitosterol adsorbed, q_e (mg/g)
NIP_TFMAA-co-TRIM	0.9169
MIP_TFMAA-co-TRIM	1.1171

It is clear that MIP_TFMAA-co-TRIM can adsorb more β-sitosterol than NIP_TFMAA-co-TRIM. This happens because cavities in MIP with the presence of functional groups of O-H, C = O, and C-F can easily recognize β-sitosterol and the functional groups can interact with the O-H group of β-sitosterol to form hydrogen bonds.

The optimum pH was determined by studying the effect of different pH on the adsorption of β-sitosterol by MIP_TFMAA-co-TRIM. Figure 3 shows the amount of β-sitosterol adsorbed by MIP_TFMAA-co-TRIM as a function of pH.

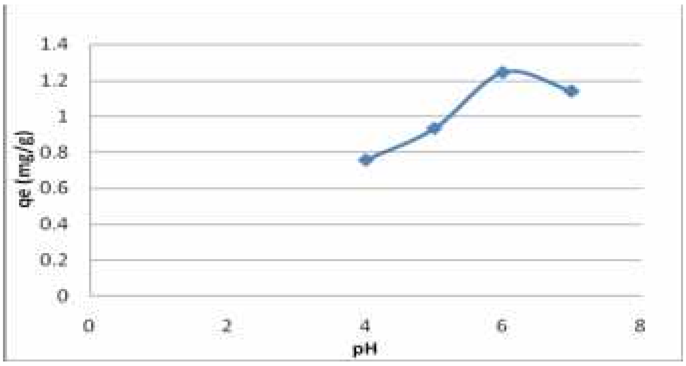


Figure 3 pH optimum curve of MIP_TFMAA-co-TRIM

It is obvious that the amount of β-sitosterol adsorbed by MIP-TFMAA-co-TRIM at a pH of 4 is low. This because at a low pH, the concentration of H^+ ions in the solution is high. Therefore, the protonation of functional groups on the adsorbent will occur. As a consequence, there will be electrostatic repulsion between adsorbent and the target molecule and the amount of β-sitosterol adsorbed is low. The amount adsorbed increases with the increase of pH until achieve the highest amount adsorbed at a pH of 6. The formation of hydrogen bonds between the hydroxyl functional groups on the β-sitosterol and carbonyl or hydroxyl functional groups on monomer TFMAA increased at the optimum pH because the amount of H^+ ions in the solution decreases. When the pH increased to a pH of 7, β-sitosterol became more neutral. Therefore hydrogen bonding interactions with the polymer became weak.

Figure 4 shows that the amount of adsorbed by MIP_TFMAA-co-TRIM is different every time. The amount-adsorbed increases with time until an equilibrium was reached in 60 minutes then decreases at longer time because the adsorbent surface has been saturated by β-sitosterol.

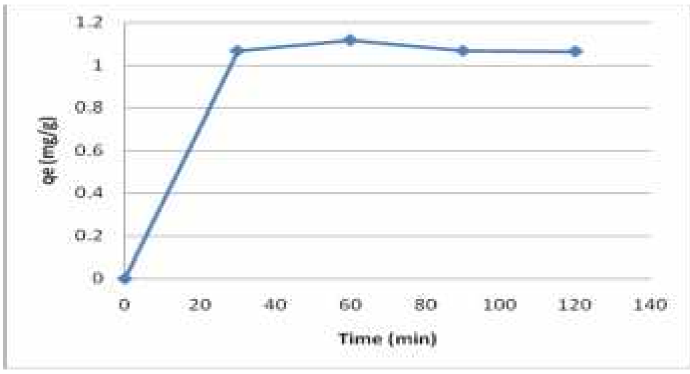
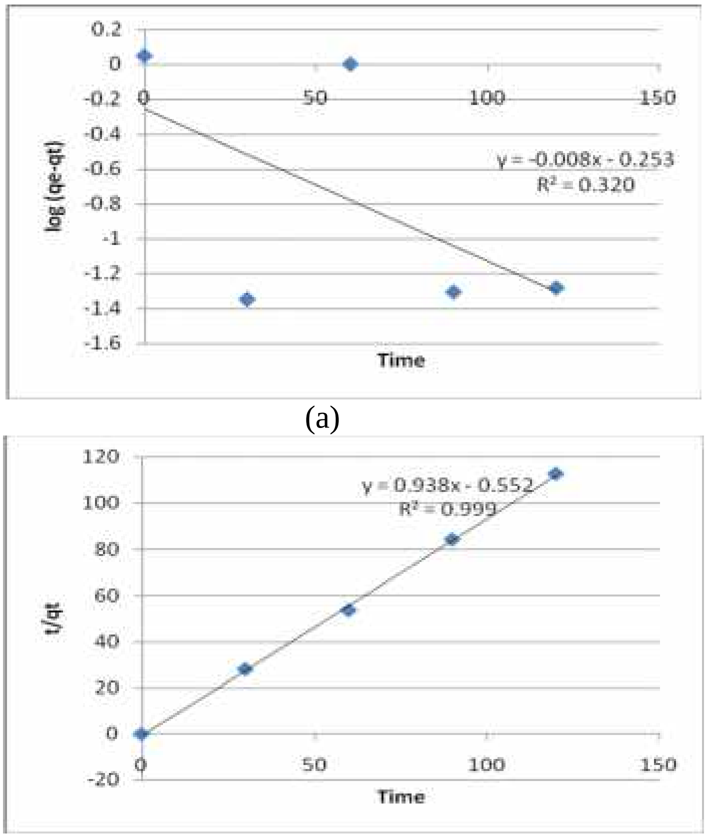


Figure 4. Curve of optimum time MIP_TFMAA-co-TRIM

Adsorption kinetics for MIP_TFMAA-co-TRIM was analyzed using a pseudo first order and pseudo second order models. Values of R_2, k_1, k_2, q_e obtained from calculation are presented in Table 2



(a)

(b)

Figure 5. Adsorption kinetic obtained from using a) pseudo first order, and b) pseudo second order equations.

Table 2 Kinetics Parameter for MIP_TFMAA-co-TRIM

Adsorption kinetic models	k_1	k_2	calculated q_e (mg/g)	experimental q_e (mg/g)	R^2
One pseudo-order	0.0184	-	0.5585	1.1171	0.320
Pseudo second order	-	1.7895	1.0661	1.1171	0.999

Figure 5 illustrates that the adsorption kinetic of pseudo second order provides data correlation better than that of pseudo first order and Table 2 shows that value of R^2 for kinetic model of pseudo second order close to 1, while the kinetic model of pseudo first order is far from 1.

Value of q_e calculated from equations of pseudo first order and pseudo second order are 0.5585 and 1.0661, respectively, while the q_e values obtained from the experiment is 1.1171 mg/g. It is clear that the q_e value obtained using the pseudo second order model is close to the experimental value of q_e, so it can be concluded that the pseudo second order model is more suitable as adsorption kinetics model for MIP-co-TRIM than a pseudo first order. Adsorption kinetics model of pseudo second order indicates that chemisorption (chemical adsorption) involves in the β-sitosterol adsorption.

Conclusion

Functional groups that involve in the formation of MIP_TFMAA-co-TRIM were O-H, C=O, C=C, and C-F. The surface of MIP is rougher and more porous than that of NIP. MIP was able to adsorb β-sitosterol better than the NIP in which the amount of β-sitosterol adsorbed was 1.1171 mg/g, while NIP_TFMAA-co-TRIM were 0.9169 mg/g. The optimum pH and time for the adsorption of β-sitosterol by MIP were 6 and 60 minutes, respectively. Adsorption kinetics followed the model of pseudo second order kinetics.

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