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Synthesis of Moleculary Imprinted Polymer Originated from TFMAA and TRIM for Sterol Separation

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Molecular imprinted polymer (MIP) was synthesized bulk polymerization using 2-(trifluoromethyl)acrylic acid (TFMAA) as a functional monomer, trimethylolpropane trimethacrylate (TRIM) as a cross-linker, benzoyl peroxide as an initiator, acetone as porogen solvent, and stigmasterol as template molecule to obtain adsorbent having high affinity and selectivity to stigmasterol for separation of stigmasterol or other sterols in liquid phase. Functional groups and the morphology of the obtained MIP were investigated by Fourier transform infrared (FT-IR) and scanning electron microscope (SEM), respectively. The performance in sterol adsorption of MIPs synthesized under various conditions was investigated using a model solution of sterol mixture in n-heptane with initial concentration of 1.40×10^{-4} kg/kg-solution comparing with non-imprinted polymer (NIP). Adsorbent (1 wt%) was added to model solution and shaken in an orbital shaker at 3.67 rps, 303 K for 2.16×10^{4} s. The analysis of variance (ANOVA) suggested that the cross-linker was more influential factor on the adsorption performance of MIP as compared to the template molecule and solvent. The optimization showed that MIP synthesized at 0.5×10^{-3} mol of cross-linker, 1.0×10^{-4} mol of template molecule, and 1.0×10^{-5} m^3 of solvent had highest percentage adsorption of 57.73 % which was 1.37 times of NIP.

1. Introduction

Sterols are generally found in small amounts naturally in many vegetable oils (Phillips et al., 2002) and deodorizer distillate (DD) by-product of deodorization (Gunawan and Ju, 2009). It was employed as starting material in food, cosmetics and pharmaceutical industries (Fernandes and Cabral, 2007). However, it is usually difficult to be separated from other compounds. In recent year, two main processes have been applied for the recovery of sterol from DD including chemical and physical treatments. In the first approach, FFA in DD was saponified and the resulted soap was removed from the obtained mixture by simple solid-liquid separation. In the last step, sterols were separated from the liquid mixture of unsaponifiable components by using either vacuum distillation (Rohr, 2003) or cold crystallization (Khatoon et al., 2010). In the second approach, FFA and glycerides were chemically transformed to fatty acid alkyl esters (FAAE) by esterification (Moreira and Baltanás, 2004) or esterification followed by transesterification (Wollmann et al., 2005). Then, vacuum distillation was applied to remove large fraction of FAAE. Similar to the former approach, cold crystallization was also applied as the last step of sterol isolation. However, the major problem of these processes was the high energy requirement. In general, vacuum distillation was operated at 100 to 133.32 Pa and 453 to 473 K to remove undesired compounds in the mixture from chemical treatment, and cold crystallization was used to separate sterol from the remaining mixture at low temperature between 253 to 288 K. Therefore, a method with high efficiency and more economical value should be developed to serve rapid growth of recent sterol demand.
Adsorption process with activated carbon (Barder, 1989) and magnesium silicate (Barder et al., 1990) were used to recover sterol from natural resources. However, these adsorbents have shown some significant disadvantages, which include high capital costs and low selectivity to sterol molecule. Therefore, it is necessary to develop the highly selective and economical adsorbent material for the recovery of sterol. Polymeric materials have been used in many applications in the field of chemistry and engineering such as separation processes (Ahmad et al., 2015), catalyst based (Protsenko et al., 2016), and biosensor (González-Delgado et al., 2016).

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Molecular imprinted polymer (MIP) is one alternative technique to prepare the adsorbent with selective molecular recognition ability. This technique requires the template molecule to create cavities in polymer matrix after removal of template molecule.

In this study, MIPs were synthesized using 2-(trifluoromethyl)acrylic acid as a functional monomer, trimethylolpropane trimethacrylate as a cross-linker, benzoyl peroxide as an initiator, acetone as a porogen solvent, and stigmasterol as a template molecule to obtain adsorbent having high selectivity to stigmasterol for separation of stigmasterol or other sterols in liquid phase. Analysis of variance (ANOVA) was used to determine the effect of three synthesis factors of MIP such as amount of cross-linker, template molecule, and solvent for percentage adsorption of sterol. Functional groups and the morphology of MIP were investigated by Fourier transform infrared (FT-IR) and scanning electron microscope (SEM), respectively. Batch adsorption was performed to evaluate the performance of the obtained MIPs in sterol adsorption using a model solution of sterol mixture in n-heptane comparing with non-imprinted polymer (NIP).

2. Materials and Methods

2.1 Materials

2-(trifluoromethyl)acrylic acid (Alfa Aesar) as a functional monomer, trimethylolpropane trimethacrylate (Sigma-Aldrich) as a cross-linker, benzoyl peroxide (Merck) as an initiator, stigmasterol (Tama Biochemical) as a template molecule, and acetone (Asian scientific) as a porogen solvent. Sterol mixture (Acinopeptide) consisted campesterol (23.6 wt %), stigmasterol (28.2 wt %), and β-sitosterol (48.2 wt %) was used as an adsorbate. N-heptane (Apex Chemicals) was used as a model solution.

2.2 Synthesis of molecular imprinted polymer

2-(trifluoromethyl)acrylic acid (TFMAA), trimethylolpropane trimethacrylate (TRIM), stigmasterol and acetone were mixed in 6.0×10^{-5} m^3 glass bottle. A nitrogen gas was flowed into the mixture for 600 s to remove oxygen. Then the mixture was shaken by an orbital shaker at 3.67 rps and room temperature to form a homogeneous solution. After that, the solution was polymerized at 333 K by shaking at 3.67 rps for 8.64×10^{4} s. After polymerization process, the obtained solid polymer was ground by ceramic mortar. The obtained polymer powder was washed several times with distilled water to remove all unreacted reagent. The distillated water was separated from the polymer powder by centrifugation at 66.67 rps for 1.8×10^{3} s and the water was taken to analyze UV-absorption of residual excess unreacted reagent using UV-spectrophotometer. The polymer powder was dried at 383 K for 8.64×10^{4} s. To remove the template molecule from polymer powder, soxhlet extraction was used at 393 K for 8.64×10^{4} s with solution consisting of acetonitrile (85 vol%), methanol (5 vol%), and water containing 1 vol% of acetic acid (10 vol%). Different MIPs were synthesized by varying three independent factors such as amount of cross-linker (A), template molecule (B), and solvent (C) in the range of 0.5×10^{-3} to 1.5×10^{-3} mol, 1.0×10^{-4} to 1.5×10^{-4} mol, and 1.0×10^{-5} to 2.0×10^{-5} m^3, respectively. Amount of functional monomer and initiator were fixed at 1.0×10^{-3} and 8.5×10^{-5} mol, respectively. Conditions for MIPs synthesis were determined using central composite design (CCD) as shown in Table 1. Non-imprinted polymer (NIP) was synthesized without a template molecule with the same synthesis procedures as MIP.

Table 1: Conditions for MIPs synthesis determined by central composite design (alpha value = 2.0)

No.	Cross-linker $\times10^{3}$ (mol)	Template molecule $\times10^{4}$ (mol)	Solvent $\times10^{5}$ (m^3)
1	0.75	1.125	1.25
2	1.25	1.125	1.25
3	0.75	1.375	1.25
4	1.25	1.375	1.25
5	0.75	1.125	1.75
6	1.25	1.125	1.75
7	0.75	1.375	1.75
8	1.25	1.375	1.75
9	0.50	1.250	1.50
10	1.50	1.250	1.50
11	1.00	1.000	1.50
12	1.00	1.500	1.50
13	1.00	1.250	1.00
14	1.00	1.250	2.00
STD	1.00	1.250	1.50

2.3 Characterization of molecular imprinted polymer

Morphology of MIP and NIP was investigated by Scanning electron microscope (SEM) using EVO®MA10 (ZEISS). Extra high-tension voltage level was 1.5×10^{4} V. Functional groups of MIP and NIP were investigated by Fourier transform infrared (FT-IR) using IRPrestige-21 (Shimadzu, Japan) equipped with MIRacle ATR (PIKE Technologies, Inc.) with a resolution of 4 cm^{-1}.

2.4 Adsorption performance test

The adsorption performance of MIPs synthesized under various conditions was investigated using a model solution of sterol mixture in n-heptane with initial concentration of 1.40×10^{-4} kg/kg-solution comparing with NIP. Adsorbent (1 wt%) was added to model solution and shaken in an orbital shaker at 3.67 rps, 303 K for 2.16×10^{4} s. Sample before and after adsorption were taken for quantitative analysis of campesterol, stigmasterol, and β-sitosterol. Analysis was performed using a gas chromatography connected with flame ionized detector (GC-2010plus; Shimadzu). Peak separation was achieved using a ZB-5HT capillary column (30 m in length, 3.2×10^{-4} m in internal diameter, 1.0×10^{-7} m in film thickness; Phenomenex). Tricaprin was used as an internal standard. Adsorption performance was evaluated by the percentage adsorption of sterol (% Ads), adsorption capacity (q), and selectivity base on stigmasterol (S_i) were calculated according to Eq(1), Eq(2), and Eq(3).

$$\%\,Ads = \frac{C_0 - C}{C_0}\times 100 \tag{1}$$

$$q = \frac{(C_0 - C)W_{sol}}{W_{ads}} \tag{2}$$

$$S_i = \left(\frac{W_{i,0} - W_i}{W_{i,0}}\right)\left(\frac{W_{stigma,0}}{W_{stigma,0} - W_{stigma}}\right) \tag{3}$$

Where C_0 and C are liquid-phase concentration of stigmasterol at initial and at time t, respectively. W_{sol} is the weight of the solution, and W_{ads} is the weight of adsorbent used. $W_{i,0}$ and W_i are the weight of species "i" at initial and at time t, respectively. $W_{stigma,0}$ and W_{stigma} are the weight of stigmasterol at initial and at time t, respectively.

2.5 Statistical analysis

The percentage adsorption of sterol was selected as the response factor, and the relationship between response factor and independent factors was approximated by quadratic model equation. The statistical of fitted quadratic model was analyzed by analysis of variance (ANOVA). The quality of fitted quadratic model was expressed by coefficient of determination (R^2), and the significant terms of model were evaluated based on the p-value with 95 % confidence.

3. Results and Discussion

3.1 Polymer characterization

The functional groups on both of polymers were explained by FT-IR spectra as shown in Figure 1. Both of NIP and MIP absorption peaks were similar and had small sharp peak of C-H vibration at 2,960 cm^{-1}, large sharp peak of C = O vibration at 1,710 cm^{-1} and the peak of C-F appeared over a very broad range of 1,000–1,400 cm^{-1}. These peaks were identified as the groups C - H, C = O, and C = F on TFMAA (Fauziah et al., 2015).

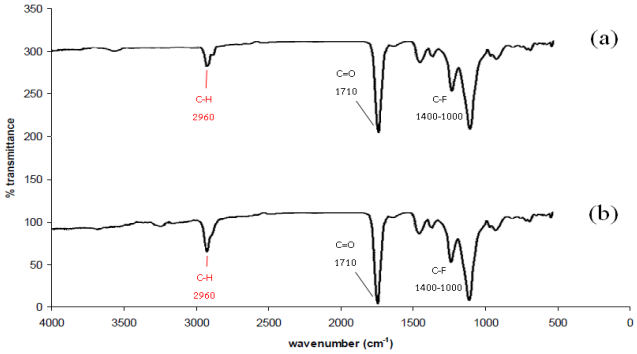

Figure 1: FT-IR spectra of (a) NIP and (b) MIP

However, absorption peak of O-H was not found on both FT-IR spectra. It is possible that O-H group undergo interaction with other functional groups. The comparison of spectra between NIP and MIP indicated that intensity of C-H peak on the NIP spectra was less than MIP spectra. It is possible that the residue of stigmasterol contained in MIP. The surface of MIP synthesized at condition number 9 (Figure 2(b)) was rougher than NIP, see Figure 2(a). Furthermore, the porous of MIP was larger than NIP. It suggested that the imprinted of stigmasterol created additional pores in polymer matrix.

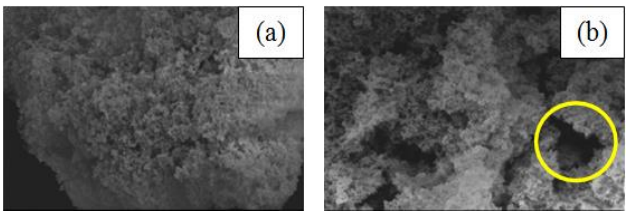

Figure 2: SEM images at a magnification of 20,000x of (a) NIP and (b) MIP

3.2 Adsorption performance

The adsorption performance of adsorbents was investigated using a model solution of sterol mixture (campesterol, stigmasterol, and β-sitosterol) in n-heptane. MIPs were synthesized under various conditions to use in the sterol adsorption comparing with NIP. Figure 3 showed the adsorption performance of MIPs comparing with NIP. MIP synthesized at condition number 9 (0.5×10^{-3} mol of cross-linker, 1.25×10^{-4} mol of template molecule, and 1.5×10^{-5} ml of solvent) adsorbed greater amount of sterol comparing with NIP. The adsorption capacities of MIP synthesized at condition number 9 and NIP were 5.93×10^{-3} and 5.85×10^{-3} kg/kg-adsorbent, respectively. However, the adsorption result revealed that all of MIPs synthesized adsorbed campesterol, stigmasterol, and β-sitosterol with the same percentage of initial amounts in model solution ($S_{campesterol} = S_{\beta\text{-}sitosterol} = 1.0$). No specific selectivity for stigmasterol adsorption should be result from very similar structure of these sterols. Based on the adsorption capacity, the result indicated that the maximum sterol adsorption capacity of MIPs (q = 5.93×10^{-3} kg/kg-adsorbent) was 5.3 times higher than the capacity of MIP synthesized using TFMAA as a functional monomer, TRIM as a cross linker and β-sitosterol as a template molecule of some reported (Fauziah et al., 2015). Therefore, it was thus a promising adsorbent and could be used for recovery of sterol by adsorption method.

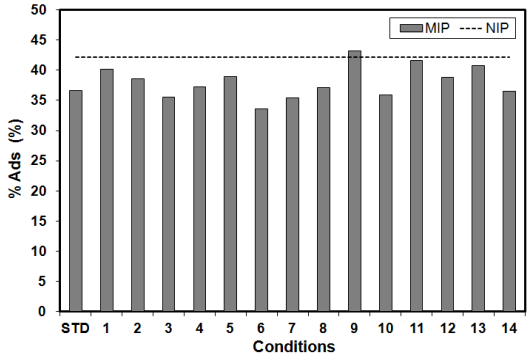

Figure 3: Adsorption performance of MIPs comparing with NIP at 303 K for 2.16×10^4 s

3.3 Statistical analysis

The results of the percentage adsorption of sterol by MIPs synthesized under various conditions were selected to analyze in order to identify significant effect of factors on the percentage adsorption of sterol by synthesized molecular imprinted polymer. The analysis of variance of percentage adsorption was summarized in Table 2. The p-value showed that the linear terms (A, B, and C), quadratic terms (A×A, B×B, and C×C) and interaction terms (A×B, A×C, and B×C) were insignificant (p-value > 0.05). It seems that all terms had no effect on the adsorption performance of MIP. However, a regression model was performed in order to obtain a suitable prediction model for percentage adsorption of sterol, and the relationship between the percentage adsorption and the independent factors was shown in Eq(4).

$$\%\,Ads = 2860 - 74.7A - 257.8B - 58.4C + 14.8A^2 + 70.3B^2 + 11.0C^2 + 41.3AB - 7.4AC + 23.2BC \quad (4)$$

Table 2: The analysis of variance of percentage adsorption of sterol by MIP

Terms	Coefficient	S.E. coefficient	p-value
Constant	286.04	1.765	0.000
Cross-linker (A)	-74.68	0.500	0.070
Template (B)	-257.78	0.500	0.210
Solvent (C)	-58.37	0.500	0.121
A × A	14.80	0.601	0.185
B × B	70.30	0.601	0.127
C × C	10.99	0.601	0.305
A × B	41.34	0.708	0.127
A × C	-7.46	0.708	0.539
B × C	23.28	0.708	0.351

The fit of model was checked by the coefficient of determination (R^2), which was calculated to be 79.39 % indicating that 79.39 % of the variability in the response could be explained by the model. Figure 4(a), 4(b), and 4(c) show the three-dimensional response surface which were shown the effect of MIP synthesis factors on the percentage adsorption of sterol. Figure 4(a) illustrated the effect of amount of template molecule and solvent on the percentage adsorption of sterol, with amount of cross-linker was fixed at 1.0×10^{-3} mol. Figure 4(b) illustrated the effect of amount of cross-linker and solvent on the percentage adsorption of sterol, with amount of template molecule was fixed at 1.25×10^{-4} mol, and Figure 4(c) illustrated the effect of amount of cross-linker and template molecule on the percentage adsorption of sterol, with amount of solvent was fixed at 1.5×10^{-5} m^3.

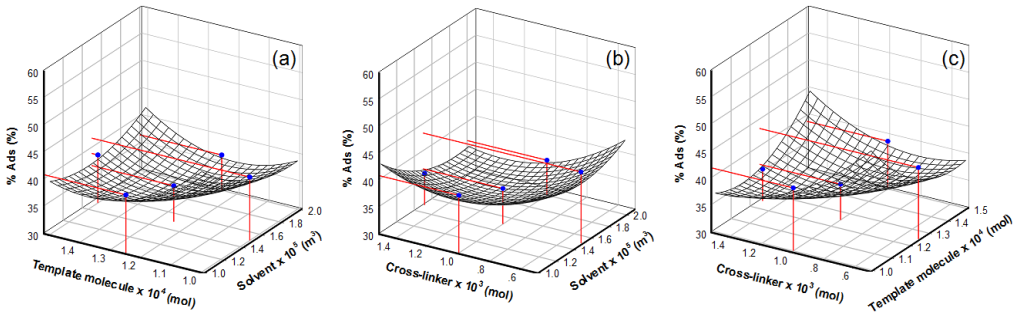

Figure 4: Three-dimensional response surface plot of the percentage adsorption of sterol by MIP; (a) effect of amount of template molecule and solvent, amount of cross-linker = 1.0×10^{-3} mol, (b) effect of amount of cross-linker and solvent, amount of template molecule = 1.25×10^{-4} mol, (c) effect of amount of cross-linker and template molecule, amount of solvent = 1.5×10^{-5} m^3 comparing with the experimental data (blue circle symbol)

The results indicated that the prediction result of the percentage adsorption of sterol was in good agreement with the experimental values. The percentage adsorption of sterol by MIP was found to decrease with increasing amount of cross-linker as shown in Figure 4(b) and 4(c). It can be seen that amount of cross-linker was more influential factor on the adsorption performance of MIP as compared to the other two factors. The high amount of cross-linker inhibited the diffusion of the template reducing the efficiency of the imprinting process (Rechichi et al., 2007). In addition, the amount of cross-linker can control the degree of MIP swelling. The rigidity of MIP is a consequence of the excess of cross-linking agents. The rigidity makes very difficult to remove template molecule from MIP, it results in the number of molecular recognition sites becomes less than the number of sites expected from the amount of template molecule used due to some template molecule are embed in the polymer matrix (Park et al., 2005). These results were presented by the case of the amount of template molecule in the range of 1.0×10^{-4} to 1.35×10^{-4} mol as shown in Figure 4(C), the MIP synthesized with 0.5×10^{-3} mol of cross-linker exhibited higher percentage adsorption than that 1.5×10^{-3} mol of cross-linker. However, MIPs synthesized with 0.5×10^{-3} mol of cross-linker exhibited low percentage adsorption when increasing amount of template molecule. It is possible that the excess amount of template molecule interacted with the functional monomer as well as with the cross-linker. This could reduce the number of interaction between the functional monomer and cross-linker which could generate fewer effective cavities in the polymer matrix and reduce the number of molecular recognition sites. According to the optimization, the result showed that MIP synthesized at 0.5×10^{-3} mol of cross-linker, 1.0×10^{-4} mol of template molecule, and 1.0×10^{-5} m^3 of solvent had highest percentage adsorption of 57.73 % which was 1.37 times of NIP.

4. Conclusions

Molecular imprinted polymers (MIPs) were synthesized by bulk polymerization using TFMAA as a functional monomer, TRIM as a cross-linker, benzoyl peroxide as an initiator, acetone as porogen solvent, and stigmasterol as a template. The analysis of variance (ANOVA) suggested that cross-linker was more influential factor on the adsorption performance of MIP compared to template molecule and solvent. The optimization showed that MIP synthesized at 0.5×10^{-3} mol of cross-linker, 1.0×10^{-4} mol of template molecule, 1.0×10^{-5} m^3 of solvent had the highest adsorption capacity of 57.73 % (1.37 times of NIP). Moreover, adsorption was successfully performed at 303 K. In considering energy requirement for adsorption compared with vacuum distillation at temperature of 453 to 473 K or cold crystallization at 253 to 288 K, adsorption is promising method for sterol recovery in large scale with fewer energy requirement.

Acknowledgments

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