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Molecularly Imprinted Polymers as β -sitosterol selective adsorbent using combination of Methacrylate Acid and Trimethylpropane Trimethacrylate

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Abstract : The research aim was to synthesize Molecularly Imprinted Polymers (MIP) that has a memory effect on β - sitosterol. The synthesis was conducted through ²¹ bulk polymerization process using a methacrylic acid monomer (MAA), trimethyl propane trimethacrylate (TRIM) as a cross linker and β -sitosterol as a template. The MIP was used as a selective adsorbent of β -sitosterol. Characterization of MIP was performed using FTIR, SEM, and TGA. The β -sitosterol adsorption ability of MIP optimized at various pH and time. The amount of β -sitosterol adsorbed by MIP was analyzed using HPLC. The FTIR spectrum shows that functional groups that play a role in MIP formation are OH, C=C and C=O. The MIP morphological shape was spherical like a ball with a particle diameter size of about 2.5 μ m and the surface of MIP was rougher than NIP. The analysis results of HPLC proven that MIP was able to adsorb β -sitosterol better than NIP.

Key words : Synthesis, MIP, TRIM, MAA, β -sitosterol, cross-linker.

Introduction

The separation and purification of a chemical compound from natural product remains an obstacle because the complexity of components in the sample extract. The important step in the separation process was extraction, but it takes a long time and expensive. The best and most efficient way to solve this problem was a selective extraction to a target compound and important to be separated¹, however the selective extraction of a compound in the sample requires selective materials in the form of synthetic polymers. The synthetic polymers can be prepared by polymer imprinting techniques through free radical polymerization reactions to produce molecularly imprinting polymers (MIPs)¹³.

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The MIPs are very stable at various pH, solvent, temperature, and very selective to target molecules^{6,3} and can also be used more than once¹⁷. Currently, MIP applications are increasing in the fields of chemistry, biology, health and environment^{11,12} especially as adsorbent or stationary phase for the separation of compounds, as biosensor and drug material^{9,11,12}. In the field of chemistry, MIP was widely used as a material for the separation of amino acid compounds, isomers and enantiomer monomers from natural material samples². The MIP as a selective adsorbent can be produced using appropriate functional monomers and cross-linkers. The monomers are important to determine the ability of MIP to interact selectively with target molecules⁷. Application of appropriate cross-linkers can determine the stability of MIP formed during the polymerization process¹.

In a previous study, the use of a MAA monomer and an EGDMA cross-linker (MIP_MAA-co-EGDMA) has shown better adsorption and stability capabilities¹⁰ than the MIP using a trifluoro methylacrylic acid (TFMAA) monomer and the EGDMA cross-linker (MIP_TFMAA-co-EGDMA)⁴. The condition was affected by steric and induction effects of the fluorine (F) group on the TFMAA monomer, so that the binding ability between the monomers and the cross-linkers reduces. The MIP used a TRIM cross-linker combined with a TFMAA monomer (MIP_TFMAA-co-TRIM) had a better adsorption capability and were more stable than MIP synthesized using TFMAA monomer combined with EGDMA cross linking (MIP_TFMAA-co-EGDMA)⁴. TRIM is a superior cross-linker compared to EGDMA cross-linkers because it has more vinyl clusters which contribute to binding with more monomers so that the resulting polymer is more stable and has a high degree of stiffness¹⁴.

Therefore, the proper selection of monomers and cross-linkers are necessary. In this study, the template molecules used were β -sitosterol compounds. The target molecule is one of plant and animal steroid compounds that often interfere in the process of separating and purifying certain compounds in natural products¹⁵. Therefore β -sitosterol was selected as a template molecule in MIP synthesis as a selective adsorbent of β -sitosterol.

Experimental

Material

The materials used in the study consisted of methacrylic acid 99% (Aldrich sigma), β -sitosterol 97% (Aldrich sigma), trimethyl propane trimethacrylate 98% (Aldrich sigma), toluene (E. Merck) as porogen solvent, 2, 2'-azobisisobutyronitril (Sigma Aldrich) as the initiator, tetrahydrofuran (THF) (E. Merck) and acetic acid (E. Merck). Double distilled water and methanol (HPLC grade) were used in HPLC analysis. Equipment used include stirrer, glass equipment, digital balance, water bath, oven, HPLC instrument of Agilent 1260 infinity with the column type Cronus RP E18C and column length 12.5 cm x 0.4 cm, FTIR Shimadzu type IRPrestige-21, and SEM of type Vega 3SB and EDS 6510 (LA, and TGA NETZ.SCH STA 449F).

Synthesis of MIP_MAA-co-TRIM

In the MIP synthesis, 2 ml of MAA functional monomer and 0.05 g of β -sitosterol were dissolved in a round bottom flask containing 10 ml (9.41 mmol) of toluene. After being dissolved, 3 ml (9.3 mmol) of TRIM and 0.05 g (0.3 mmol) AIBN were added. The solution was sonicated and nitrogen gas was flowed into the solution to remove dissolved oxygen. The polymerization process was carried out in the water bath at 55°C for 24 hours. The synthesized polymer was dried, and then washed with THF, methanol/acetic acid (9:1), and double distilled water to remove the template, porogenous solvent and other compounds. The washed polymer was dried at 50°C. The Non-imprinting polymer (NIP) was made without the molecular template with the same synthesis procedure as MIP¹³.

Characterization of MIP_MAA-co-TRIM and NIP_MAA-co-TRIM

Characterization of MIP_MAA-co-TRIM and NIP_MAA-co-TRIM uses several instruments including Scanning Electron Micrographs (SEM) for surface and morphological characteristics of the shape and size of the object particles, Energy Dispersive X-ray Spectroscopy (EDS) for elemental analysis, Fourier Transform Infrared (FTIR) to determine active functional groups and Thermo gravimetric Analysis (TGA) to determine the thermal stability of MIP_MAA-co-TRIM and NIP_MAA-co-TRIM.

The pH effect on MIP_MAA-co-TRIM adsorption

The total of 20 mg of MIP_MAA-co-TRIM was put into four vials and then added 3 ml of 10 ppm β -sitosterol solution with pH variation of 4, 5, 6, and 7. The solution was shaken at room temperature for 6 hours and then filtered. The concentration of β -sitosterol in the filtrate was analyzed by a UV spectrophotometer at a wavelength of 202 nm

The effect of contact time on adsorption of MIP_MAA-co-TRIM

The each of five vials was filled with 20 mg MIP_MAA-co-TRIM then added 3 ml of 10 ppm β -sitosterol solution. The adsorption process was done at different time settings for 30, 60, 120, 180, 240 minutes at room temperature and then the solution was filtered. The β -sitosterol concentration was analyzed by a UV spectrophotometer at 202 nm wavelength.

The test of NIP and MIP adsorption ability

The adsorption ability of MIP_MAA-co-TRIM to the target molecule was tested. By putting 20 mg of MIP and NIP into an erlenmeyer flask then added with 3 ml of 10 ppm β -sitosterol solution. The solution was shaken for one hour at room temperature and the filtrate was analyzed by HPLC.

Result and Discussion

Synthesis MIP and NIP

Molecularly Imprinted Polymers (MIP_MAA) and Non Imprinted Polymers (NIP_MAA) synthesized were white powders.

Characterization of MIP and NIP

FTIR analysis

FTIR data from the NIP and MIP obtained can explain the functional groups that have an effect on polymer formation. Figure 1 shows that, the absorption peaks on the NIP_MAA-co-TRIM and MIP_MAA-co-TRIM (before washing) spectrums are changing when compared to the MAA monomer spectrum. The shift of the absorption peak of the OH group from MAA to NIP_MAA-co-TRIM and MIP_MAA-co-TRIM (before washing) is due to the interaction of the -OH group of the MAA monomer with the -OH functional group of β -sitosterol or -OH group between monomers to form hydrogen bonds with wave number value as given in Table 1.

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Table 1.

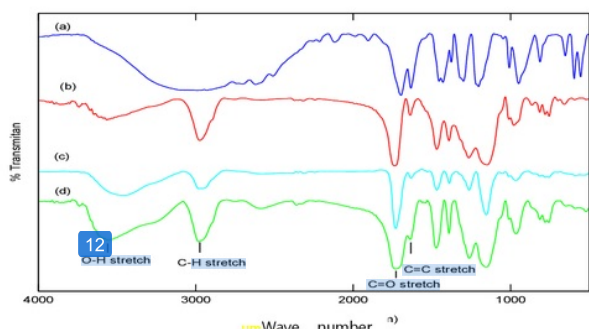


Figure 1. Spectrum of FTIR: a) MAA, b) NIP_MAA-co-TRIM, c) MIP_MAA-co-TRIM (before washed or bw) d) MIP_MAA-co-TRIM (after washing or aw)

The interaction may also occur between the -C=O functional groups in MAA and -OH monomers of β -sitosterol forming a hydrogen bond causing a shift of the wave numbers on -C=O function group in NIP_MAA-co-TRIM and MIP_MAA-co-TRIM (bw).

The wave number for -C=C functional group of MAA monomer undergoes a very small shift. However, Figure 1 shows that the intensity and the sharp of the -C=C stretching vibration of the MAA monomer reduce after the formation of NIP_MAA-co-TRIM and MIP_MAA-co-TRIM. These indicated that there were interaction between the -C=C functional group of MAA and the cross linker.

Table 1. The Absorption of functional groups of MAA, NIP_MAA-co-TRIM, MIP_MAA-co-TRIM (bw), and MIP_MAA-co-TRIM (aw).

Functional groups	Wave number (cm ⁻¹)			
	MAA	NIP_MAA-co-TRIM	MIP_MAA-co-TRIM (bw)	MIP_MAA-co-TRIM (aw)
-O-H stretching	3400-3200	3564	3460	3560
C=O stretching	1695	1735	1730	1732
C=C stretching	1633	1637	1633	1637

The wave number of the -OH stretching vibration in MIP_MAA-co-TRIM (after washing) is greater than that in MIP_MAA-co-TRIM (bw) as a result of the breaking of the hydrogen bond between the -OH functional group in the β -sitosterol with the active site of the MIP_MAA-co-TRIM (bw) template.

Binding evaluation of MIP_MAA-co-TRIM

In MIP synthesis, non-covalent interactions developed by Mosbach and Arshadyat 1981 are most commonly used because they are simple and flexible¹⁴. In non-covalent MIP synthesis, the interactions between template molecules and monomers are not strong enough to maintain the complex (weak intermolecular interaction). Therefore, excess monomers are required for complex stability⁵. The polymerization steps of MIP synthesis are the initiation, propagation and termination phase. Synthesis through the polymerization step of MIP_MAA-co-TRIM is proposed as in Figure 2.

In the pre-polymerization step (Figure 2), the monomers and molds dissolved in toluene interact in advance non-covalently by forming hydrogen bonds. The polymerization step begins with initiation by free radicals from AIBN interacting with the double bonds of TRIM to form free radicals, or interacting with the double bond of MAA to form free radicals and followed by chain extension (propagation) of the monomer or other cross linking forming new bonds.

Termination of the chain extension (termination) occurs after two radicals of the chain extension result interact. The next step is the release of β -sitosterol in order to obtain molds with functional groups corresponding to β -sitosterol as the target molecule.

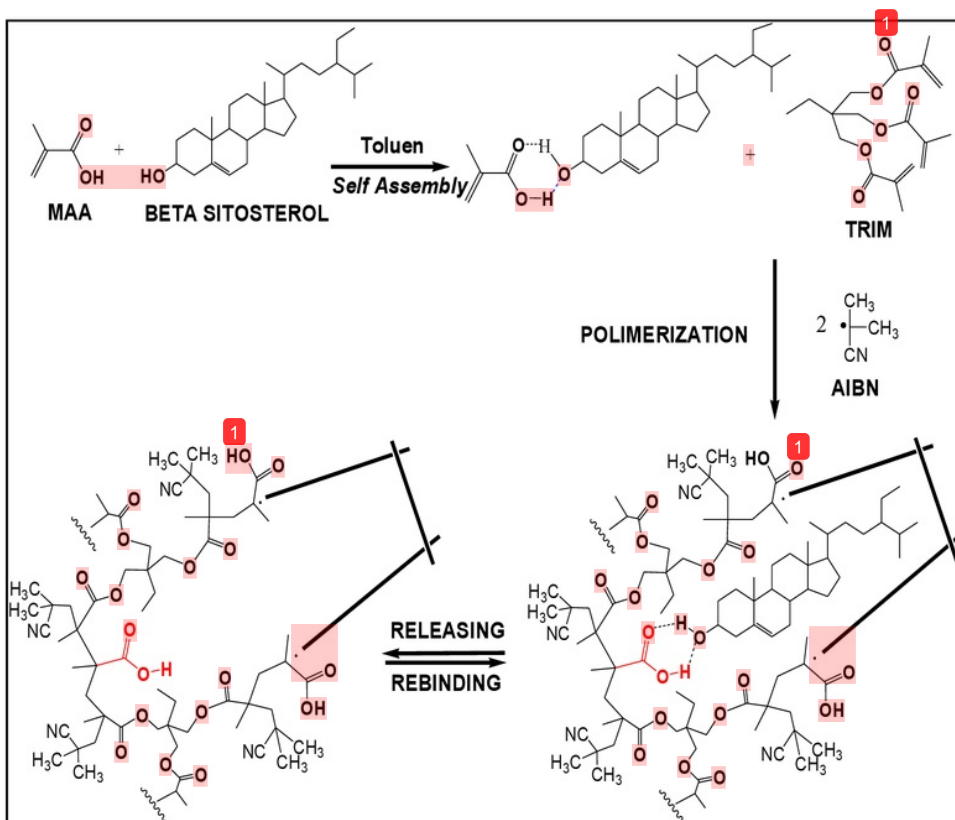


Figure 2. Proposed schemes for MIP_MAA-co-TRIM synthesis pathway

β -sitosterol may react non-covalently with the -COOH group of MIPs by hydrogen bonds due to the suitability of shape, size, and functional groups.

18

Morphological Characterization of MIP and NIP

Analysis of SEM

The result of morphological images of NIP_MAA-co-TRIM and MIP_MAA-co-TRIM using SEM can be seen in Figure 2. The surface morphology of NIP_MAA-co-TRIM is composed of an aggregate of granules with spherical form that coalesce with each other. The diameter of the granules was about 2.5 μm . The surface morphology of NIP_MAA-co-TRIM was similar to MIP_MAA-co-TRIM (bw), whereas surface morphology of MIP_MAA-co-TRIM (aw) appears to be composed of round grains of similar size and more porous, Indicates that the template molecule has been separated from MIP. The geometric structure of MIP_MAA-co-TRIM looks more rigid.

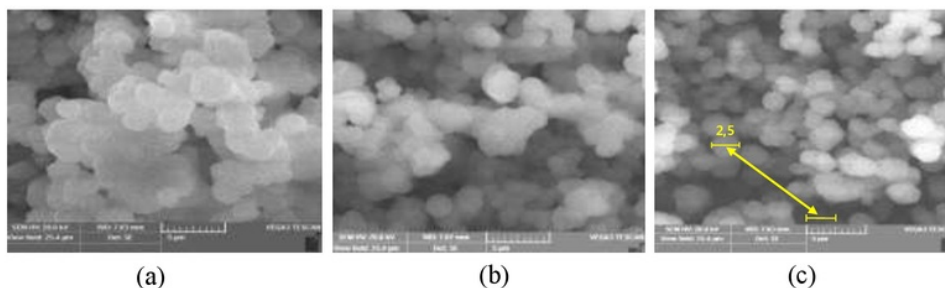


Figure 2. Morphology: a) NIP_MAA-co-TRIM, b) MIP_MAA-co-TRIM (bw), c) MIP_MAA-co-TRIM (aw)

Analysis of EDS

EDS analysis data in the Table 2 showing the composition of the main constituent elements on NIP_MAA-co-TRIM, MIP_MAA-co-TRIM (bw), MIP_MAA-co-TRIM (aw) as results of interpretation of the EDS spectrum for NIP_MAA-co-TRIM (Figure 3), MIP_MAA-co-TRIM (bw) (Figure 4) and MIP_MAA-co-TRIM (Figure 5).

Table 2. EDS data of NIP, MIP_MAA-co-TRIM(bw), MIP_MAA-co-TRIM(aw), NIP_MAA-co-TRIM

Atoms	% Mass			% Atom		
	NIP	MIP _(bw)	MIP _(aw)	NIP	MIP _(bw)	MIP _(aw)
C	79.04	80.42	77.31	83.40	84.55	81.95
O	20.96	19.58	22.69	16.60	15.45	18.05

bw: before washing, aw: after washing

The data show that the presence of β -sitosterol causes the number of C atoms to increase in MIP_MAA-co-TRIM (bw), this causes the percentage value of C at MIP_MAA-co-TRIM (bw) is higher than in MIP_MAA-co-TRIM (aw).

The number of C atoms in β -sitosterol is more than that of O, so that when β -sitosterol is still in the polymer then the character of C atom increases. While the β -sitosterol was released from the polymer, the character of the O atom increases as shown the data in Table 1. The shift of the wave number of the -OH group on the MIP_MAA-co-TRIM (TE) spectrum indicates that the hydrogen bond between the functional groups in the polymer and β -sitosterol has been broken.

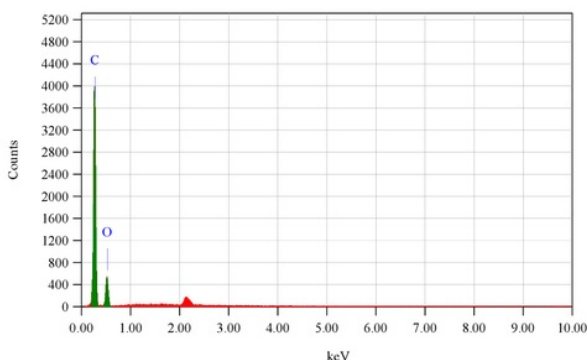


Figure 3. The EDS Spectrum of NIP_MAA-co-TRIM

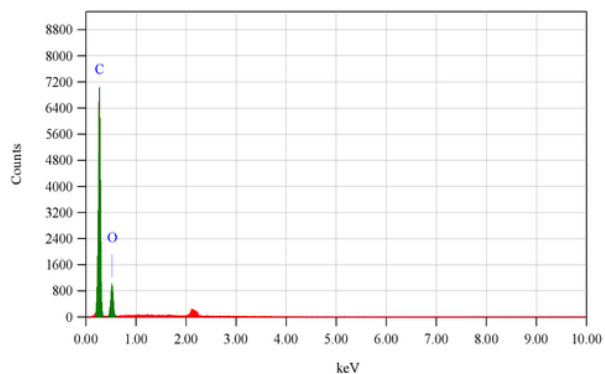


Figure 4. The EDS Spectrum of MIP_MAA-co-TRIM (before washing)

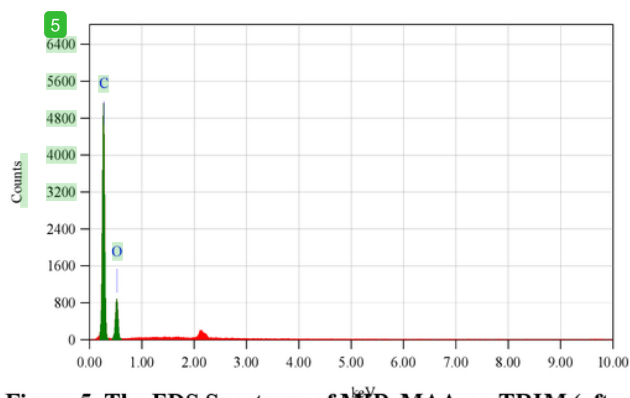


Figure 5. The EDS Spectrum of MIP_MAA-co-TRIM (after washed)

Analysis of TGA

The result of TGA analysis can be seen in Figure 6. NIP_MAA-co-TRIM thermogram has a very similar pattern to that of MIP_MAA-co-TRIM (aw). This happens because β -sitosterol is no longer exist in MIP_MAA-co-TRIM (aw) MIP_MAA-co-TRIM (bw) is different because it still contains β -sitosterol.

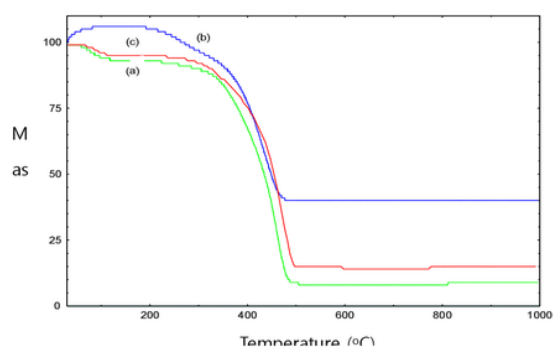


Figure 6. Thermograms of (a). NIP_MAA-co-TRIM, (b) MIP_MAA-co-TRIM (bw) and (c). MIP_MAA-co-TRIM (aw)

Based on the data derivative of TGA (DTG) NIP and MIP undergoing several time mass losses. The lost initial mass was thought to be the mass of water. Massive mass losses occur at temperatures around 402.6°C to 492.6°C for NIP_MAA-co-TRIM, temperatures of about 355.93°C to about 479.93°C for MIP_MAA-co-TRIM (bw), and above temperature 352.91°C to 482.91°C for MIP_MAA-co-TRIM (aw). At this temperature, suspected degradation occurs in MIP so that CO and CO₂ are formed. Higher temperatures cause breaking of the bond between the monomer and the cross-linker and leaving carbon. NIP and MIP began to degrade at high temperatures, indicating that MIP_MAA-co-TRIM was a stable polymer at high temperature.

Analysis of TEM

The TEM analysis results show morphological differences between MIP_MAA-co-TRIM and NIP_MAA-co-TRIM. The MIP_MAA-co-TRIM forms an aggregate composed of cavities with a pore size on a surface of about 50 nm and spherical in shape. The pores distributed on the surface can be seen in Figure 7. The cavities in MIP_MAA-co-TRIM are the spaces formed after the template removal. While NIP_MAA-co-TRIM also forms an aggregate without cavities and pores as it is made without using β -sitosterol as a cavity-printing molecule.

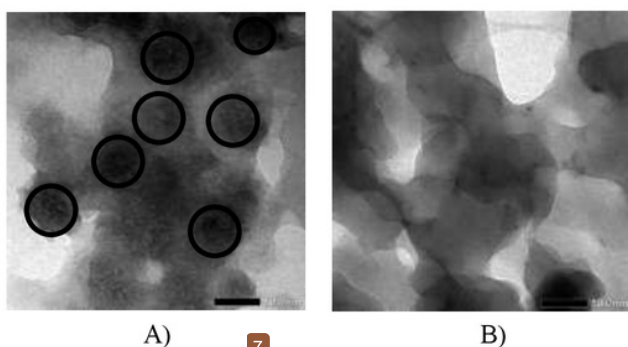


Figure 7. Morphologies of: A) MIP_MAA-co-TRIM and B) NIP_MAA-co-TRIM

The adsorption ability of MIP

The adsorption ability test was performed to determine the ability of NIP_MAA-co-TRIM and MIP_M₂₄-co-TRIM in adsorbing β -sitosterol compounds. The amount of β -sitosterol in the adsorption process by MIP can be seen in Table 1.

Table 1. The amount of β -sitosterol adsorbed by NIP_MAA-co-TRIM and MIP_MAA-co-TRIM

Kinds of polymer	The amount of β -sitosterol adsorbed (q_e)(mg/g)
NIP_MAA-co-TRIM	0.05
MIP_MAA-co-TRIM	0.75

It is clear that MIP_MAA-co-TRIM can adsorb β -sitosterol better than NIP_MAA-co-TRIM. Because of the functional groups of O-H and C=O on MIP surfaces and cavities that have an affinity for the OH functional group of β -sitosterol to form hydrogen bonds. While in NIP, the functional groups of O-H and C=O are present only on the surface.

Effect of pH on MIP_MAA-co-TRIM adsorption

The optimum pH was determined by studying the effect of pH on MIP_MAA-co-TRIM adsorption capability. The effect of pH can be seen in Figure 8.

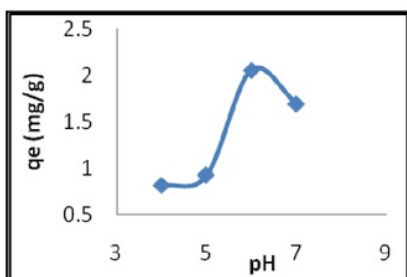


Figure 8. The pH optimum curve of MIP_MAA-co-TRIM

Adsorption of β -sitosterol on MIP-MAA-co-TRIM are to which showed optimum at pH 6 and decreased at higher pH. The adsorption capacity of MIP is low at pH below optimum because the concentration of H^+ ions in the solution is high, resulting in protonation of the functional group on the adsorbent and the hydroxyl functional group of β -sitosterol. As a result, there is an electrostatic repulsion between the adsorbent and the target molecule causing low levels of β -sitosterol adsorbed by MIP. The formation of hydrogen bonds increases between the hydroxyl functional groups in the hydroxyl group of MAA monomer at optimum pH because the concentration of H^+ ions in the solution begins to decrease. When the pH increases to pH 7, β -sitosterol is more neutral, so that the hydrogen bond interaction occurs with the active polymer.

The Influence of Time

Figure 9 shows that the adsorption of β -sitosterol on MIP_MAA-co-TRIM is different at all times. The adsorption increases with time until equilibrium is reached at 60 minutes, but decreases with increasing time. This happens because the surface of the adsorbent has been saturated by β -sitosterol.

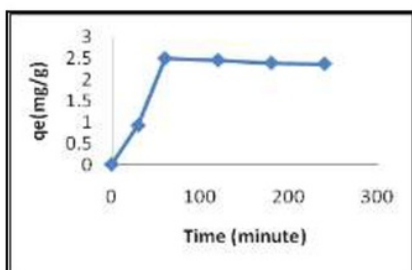


Figure 9. The amount of β -sitosterol adsorbed on MIP_MAA-co-TRIM as a function of contact times

Figure 10 shows the kinetic curves of pseudo-first order and pseudo-second order of β -sitosterol adsorption on MIP_MAA-co-TRIM.

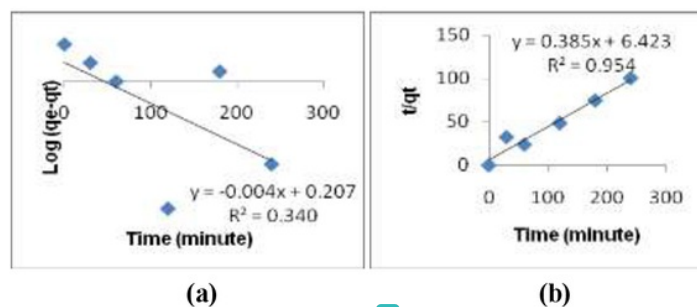


Figure 10. The Kinetic curves of (a) pseudo-order first and (b) pseudo-order second for β -sitosterol adsorption by MIP_MAA-co-TRIM

The adsorption kinetic data for MIP_MAA-co-TRIM were also analyzed using the pseudo-first order and pseudo-order second equations. The values of R^2 , k_1 , k_2 , q_e of calculation and q_e of experimental results are shown in Table 2

Table 2. The Kinetic parameter of MIP_MAA-co-TRIM

The kinetic adsorption	k_1	k_2	q_e calculation (mg/g)	q_e experiment (mg/g)	R^2
The pseudo-first order	0.0092	-	1.61	2.51	0.340
The pseudo-second order	-	0.0231	2.60	2.51	0.954

The data show that the pseudo-second order curves gives a better correlation of adsorption data than the pseudo-first order curve and the least square value (R^2) for the pseudo-second order model approaches 1, whereas that of the pseudo-first order model is not close to 1. The amount adsorbed (q_e) obtained from calculation for the pseudo-second order is closer to the experimental value. Therefore, it can be concluded that the pseudo-second order is more appropriate as the adsorption kinetics model for MIP_MAA-co-TRIM than the pseudo-first order.

Conclusion

Rigid white solids of NIP and MIP have been produced by the bulk polymerization through free radical polymerization reactions. Functional groups of OH, C=O and C=C play a role in the formation of NIP_MAA-co-TRIM and MIP_MAA-co-TRIM. MIP_MAA-co-TRIM can better adsorb β -sitosterol than NIP_MAA-co-TRIM. The surface morphology of MIP_MAA-co-TRIM was more rough and porous than NIP_MAA-co-TRIM. The optimum condition of MIP to adsorb β -sitosterol was at the pH of 6 and the time of 60 min. The adsorption of β -sitosterol on MIP_MAA-co-TRIM fitted the pseudo second order model.

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