Selectivity of betasitosterol Imprinted Polymers as Adsorbent

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Abstract. Molecularly Imprinted Polymers (MIPs) are smart materials that have been used as adsorbents in separation processes of compounds because they have a memorial effect to a certain compound. In this research, MIP synthesized was used as adsorbent for β-sitosterol. The objective of the research was to know the selectivity of MIP in adsorbing β-sitosterol. The concentrations of β-sitosterol after adsorption and desorption were analyzed by a UV-Vis spectrophotometer and the selectivity test was analyzed by HPLC. Result showed that the MIP had high adsorption ability (q_e). The recovery of β-sitosterol from MIP for the adsorption-desorption process was 68.48%. The MIP was very selective to β-sitosterol compared to cholesterol because it can adsorb β-sitosterol as many as 100%, whereas the adsorption of cholesterol was only 30.27 %.

1. Introduction

The β-sitosterol compound is one of the sterol compounds which are included in the phytosterol group [1]. The presence of sitosterol as the major compound in natural material samples often disrupts the process of isolation and purification of certain compounds [2]. This becomes one of the obstacles in the process of separation and purification of the desired compounds especially for compounds having similar structures. Therefore, one attempt to overcome this problem is to utilize the highly developed synthetic polymers as an adsorbent to assist the selective separation and purification of compounds. The material is known as molecularly imprinted polymers (MIP) [3]. The β-sitosterol compound is used as a template molecule in the MIP synthesis, in order to produce MIP that can be used as a selective adsorbent to β-sitosterol in natural material samples.

MIP is generally made through non-covalent interactions because it is simple and flexible [4]. Non-covalent interactions between monomers and templates can form the active sides of binding to MIP [5]. In addition, the release of template molecules from MIP is easier to occur due to the use of organic solvents [6]. The polymer will have a mold with the appropriate shape and size of the loose molecular template [7] and has functional groups corresponding to the target molecule [8], so that it is selective towards its target molecule.

Selective and highly adsorptive MIPs are influenced by the functional monomers and cross-linkers used. Appropriate functional monomers and cross-linking strongly determine the effectiveness of MIPs interacting with target molecules [9] and polymer complex stability formed during the polymerization process [10]. In this study, the functional monomer used was methacrylic acid (MAA).

This monomer is widely used in MIP synthesis because it ability interact with the template through hydrogen bonds and ionic bonds [11]. Cross-linkers used in this study were ethyleneglycol dimethacrylate (EGDMA). It is highly reactive and can produce polymers with high stiffness so that it is more stable [4].

2. Methods

2.1. Materials

In the research were used a functional monomer of 99% methacrylic acid, MAA, (Sigma Aldrich), template molecule of 97% β-sitosterol (Sigma Aldrich), etileneglycol dimethacrylate (Sigma Aldrich) as *cross linker,* toluene as porogen solvent, 2,2 '-azobisisobutyronitrile (Sigma Aldrich) as initiator, double distilled water, methanol (HPLC grade), tetrahydrofuran (THF), and acetic acid. Items of equipment used were glassware, shaker, digital balance, water bath, oven. Instruments used were High Pressure Liquid Chromatography (HPLC) Merck Agilent 1260 infinity (column Cronus RP E18 C, dimension 12.5 cm x 0.4 cm), Fourier Transform Infra Red (FTIR) : IR Prestige-21 (Shimadzu), Scanning Electron Micrographs (SEM) : Tescan Vega 3SB and Energy Dispersive X-ray Spectroscopy (EDS) 6510 (LA), Thermo Gravimetric Analysis (TGA) NETZSCH STA 449F1, Spectrophotometer Ultra Violet-visible (UV-Vis) Agilent 845 (Shimadzu).

2.2. Synthesis of MIP_MAA-co-EGDMA

A total 2 mL (23.58 mmol) of MAA and 50 mg (0.12 mmol) of β-sitosterol were dissolved in 5 mL (47.05 mmol) toluene into a round flask and the mixtures were left for some time. Then 3 mL (15.91 mmol) of EGDMA crosslinker and 1.5 mL of AIBN as an initiator were added to the mixture. The solution was sonicated and flowed with nitrogen gas.

Synthesis of MIP_MAA-co-EGDMA was conducted through a polymerization process for 24 hours in a water bath at a temperature of 55°C. The synthesis product was dried and then washed with tetrahydrofuran (THF), methanol/acetic acid (9:1), and double distilled water. The polymer has been washed and dried back at 50°C. Nonimprinting polymer (NIP) was made without β-sitosterol using the same synthesis procedure of MIP synthesis [12].

2.3. The Adsorption Capability Test

The adsorption ability of MIP to β-sitosterol was tested by adsorption process. The amount of β-sitosterol adsorbed by MIP was known by making various concentrations of β-sitosterol standard solutions of 2, 4, 6, 8 and 10 ppm. Five vials, each containing 20 mg of MIP, were prepared and 3 mL of β-sitosterol solution with different concentrations was added to each vial. The mixture was stirred using a shaker for 60 minutes as the optimum time [12] at room temperature, filtered and β-sitosterol concentration in the filtrate was analyzed by a UV-Vis spectrophotometer at a wavelength of 202 nm.

2.4. MIP Selectivity Test.

A minicolumn was prepared, filled with 50 mg MIP and then saturated with methanol before it was used on the selectivity test. A total of 0.5 mL of 10 ppm β-sitosterol standard solution was mixed with 0.5 mL of 10 ppm cholesterol standard solution, followed by the addition of 2 mL of a carrier carrier solvent consisting of methanol and water mixture. The mixture flowed to a minicolumn containing 50 mg of MIP. Solutions passing through the columns were collected in several vials as much as 1 mL, and 100% methanol was passed through the column. The methanol solvent that has passed through the column was also collected (1 mL each) in the vial and then the eluate was analyzed with HPLC. Results of the analysis with HPLC were calculated to determine the selectivity of MIP to β-sitosterol.

2.5. Adsorption-Desorption Test on MIP

The ability of MIP to adsorb and desorb β-sitosterol was tested using a minicolumn and a UV spectrophotometer. The minicolumn containing 50 mg of MIP was saturated with methanol. Furthermore, a standard solution of 10 ppm β-sitosterol (0.5 mL) was introduced into 2 ml of the

carrier solvent (the best solvent obtained from the selectivity test) containing methanol and water mixture. The adsorption process of β-sitosterol was carried out by flowing the mixture on the minicolumn containing 50 mg MIP. The mixture was passed through the columns and 1 mL of eluate was collected. If the carrier solvent containing β-sitosterol was discharged then 100% methanol was applied to desorb β-sitosterol which has been adsorbed by MIP. The eluate (about 1 mL each) passing through the columns was also collected and analyzed by a UV spectrophotometer.

2.6. MIP application as adsorbent by minicolu chromatography

The wood powder (50 g) was dissolved in 100 mL of n-hexane. The extraction process was carried out at temperature of room for 24 hours until obtained extract The extract was filtered and concentrated using a rotary evaporator. The jackfruit extract (10 mg) was dissolved in 10 mL of methanol and filtered. The jackfruit extract solution (1 mL) was inserted slowly into a chromatographic column which contains 100 mg of MIP. The column containing MIP was conditioned first by passing methanol several times. The β-sitosterol compound in the jackfruit extract solution is expected to be retained on MIP in the column and unexpe d components will be eluted and analyzed by HPLC. Furthermore, MIP that adsorbs β-sitosterol was washed with 1 mL of the best solvent used in the selectivity test to remove components that were not retained by MIP. The solvents that have passed through MIP in columns were collected and analyzed by HPLC. The final step was extracting the retain β-sitosterol in MIP using methanol 100%, the solvent out of column was collected as much as 1 mL and analyzed by HPLC.

3. Results and Discussion

NIP and MIP were synthesized using the bulk polymerization method through free radical polymerization reactions were white solids.

3.1. Characterization of MIP

3.1.1. Analysis FTIR. The FTIR spectra of MAA, NIP and MIP materials before and after extraction of the template can be seen in figure 1.

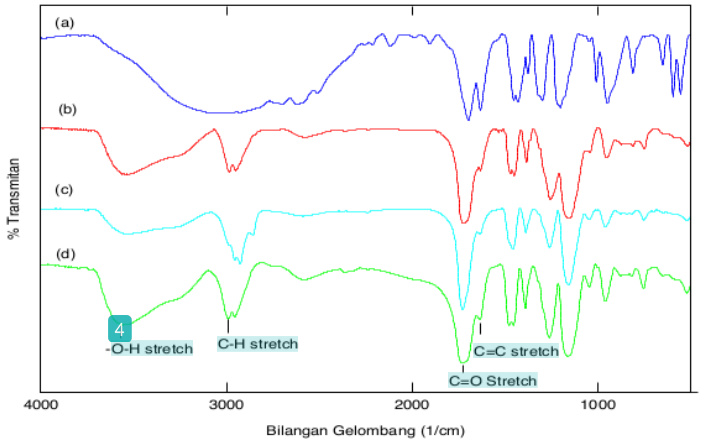


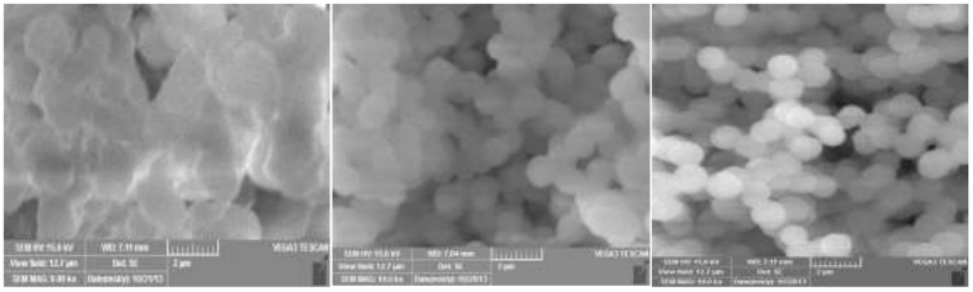
Figure 1. FTIR spectra of (a) MAA, (b) NIP_MAA-co-EGDMA (c) MIP_MAA-co-EGDMA$_{(BE)}$, (d) MIP_MAA-co-EGDMA$_{(AE)}$.

Figure 1 shows that the absorption peak of -OH stretching, C=O stretching -C=C of MAA shifts after the MAA molecule interacts with other MAA molecules, template molecules, and crosslinkers

in the formation of polymers. There is also a considerable shift of absorption peaks of -OH in NIP_MAA-co-EGDMA and MIP_MAA-co-EGDMA$_{(BE)}$ compared to that in MAA. This is because the OH functional group in the MAA molecule interacts with the functional group in the MAA itself or with OH groups of β-sitosterol that forms the hydrogen bond. Similarly, the functional groups of -C=O in MAA and OH in β-sitosterol may interact and form hydrogen bonds, this causes the wavenumber of C=O also shift after the formation of NIP_MAA-co-EGDMA and MIP_MAA-co-EGDMA$_{(BE)}$. The shift of wavenumbers for the absorption peaks of -C=C in NIP_MAA-co-EGDMA and MIP_MAA-co-EGDMA$_{(BE)}$ is very small, but there is a considerable decrease in their intensity compared to that of MAA where the -C=C- functional group have a strong and sharp absorption intensity. This suggests that the -C=C interaction of monomers occurred at the polymer formation.

The absorption peak of the -OH stretching function group on the MIP_MAA-co-EGDMA$_{(BE)}$ shifts compared to that in MIP_MAA-co-EGDMA$_{(AE)}$, this indicates that the hydrogen bond termination has occurred between -OH functional group of β-sitosterol with the functional group contained in the MIP_MAA-co-EGDMA$_{(AE)}$ template.

3.1.2. Analysis SEM. The surface morphological characteristic of NIP_MAA-co-EGDMA, MIP_MAA-co-EGDMA$_{(BE)}$ and MIP_MAA-co-EGDMA$_{(AE)}$ using SEM is shown in figure 2.



(a) (b) (c)

Figure 2. The SEM images of (a) NIP_MAA-co-EGDMA (b) MIP_MAA-co-EGDMA $_{(BE)}$ and (c) MIP_MAA-co-EGDMA$_{(AE)}$.

NIP_MAA-co-EGDMA is composed of irregularly shaped particles and they are very close each other indicating that there is no pore in the material. MIP_MAA-co-EGDMA$_{(BE)}$ is composed of spherical granules, but the particles are still close each other. By comparing with the surface morphology of MIP_MAA-co-EGDMA$_{(AE)}$, it is clear that the particles of the former are closer each other than that of the later. This indicates that before extraction of β-sitosterol, the surface is more tightly than that of MIP_MAA-co-EGDMA$_{(AE)}$ where the surface looks more porous. A more porous surface of MIP_MAA-co-EGDMA$_{(AE)}$ indicates that β-sitosterol has been detached from MIP. The SEM analysis results show that the surface of MIP_MAA-co-EGDMA$_{(AE)}$ is composed of highly regulated granules and resembles balls of similar size. All materials (NIP and MIP) looks rigid.

3.1.3. Analysis EDS. The EDS analysis was also conducted to determine the composition of the main constituent elements of each polymer. The EDS data in table 1 shows the percentage of C and O atoms in NIP and MIP materials.

Table 1. The EDS data of NIP, MIP_MAA-co-EGDMA$_{(BE)}$, MIP_MAA-co-EGDMA$_{(AE)}$.

Element	Mass %			Atomic %		
	NIP	MIP$_{(BE)}$	MIP$_{(AE)}$	NIP	MIP$_{(BE)}$	MIP$_{(AE)}$
C	82.17	79.56	77.79	85.99	83.83	82.35
O	17.83	20.44	22.21	14.01	16.17	17.65

Table 1 shows that after the extraction of β-sitosterol from MIP, the percentage of C atom decreases, while the percentage of O atom increases. This occurs because the number of C atoms in β-sitosterol is much higher than that of O atoms so that when β-sitosterol was still in the polymer, the C character increased, whereas when β-sitosterol was released from the polymer, the O character increased. A decrease in the percentage of C atom by 1.48% proves that β-sitosterol has been released from the polymer. This data is supported by the FTIR spectrum in which the absorption of the -OH functional group in the MIP_MAA-co-EGDMA$_{(AE)}$ spectrum undergoes a shift to a larger wavenumber compared to the one in the MIP_MAA-co-EGDMA$_{(BE)}$ spectrum. These results indicate that the hydrogen bonding between the functional groups of polymer and β-sitosterol broke after the removal of β-sitosterol from the MIP molecule.

3.1.4 Analysis TGA. Thermo Gravimetric analysis (TGA) was also conducted in this study to determine the thermal stability and different levels of decomposition of NIP and MIP at high temperatures. The thermogram of NIP_MAA-co-EGDMA, MIP_MAA_CO_EGDMA$_{(BE)}$ and MIP_MAA-co-EGDMA$_{(AE)}$ can be seen in figure 3.

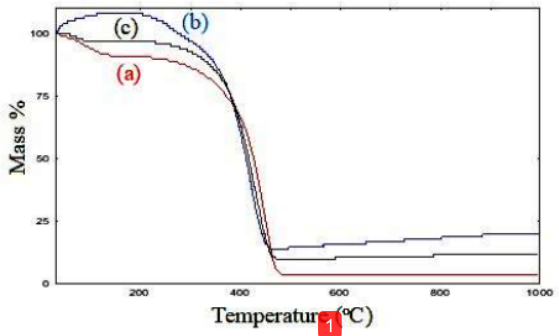


Figure 3. TGA thermograms of (a) NIP_MAA-co-EGDMA, (b) MIP_MAA-co-EGDMA$_{(BE)}$ and (c) MIP_MAA-co-EGDMA$_{(AE)}$.

The TGA thermograms of NIP_MAA-co-EGDMA and MIP_MAA-co-EGDMA$_{(AE)}$ show similar patterns because β-sitosterol in MIP_MAA-co-EGDMA$_{(AE)}$ has been removed from the polymer, while the thermogram of MIP_MAA-co-EGDMA$_{(BE)}$ is different that of the two materials because it still contains β-sitosterol.

3.1.5 *Analysis DTG.* The results of the DTG analysis (Derived Thermogravimetric Analysis) from MIP_MAA-co-EGDMA$_{(AE)}$ can be seen in figure 4.

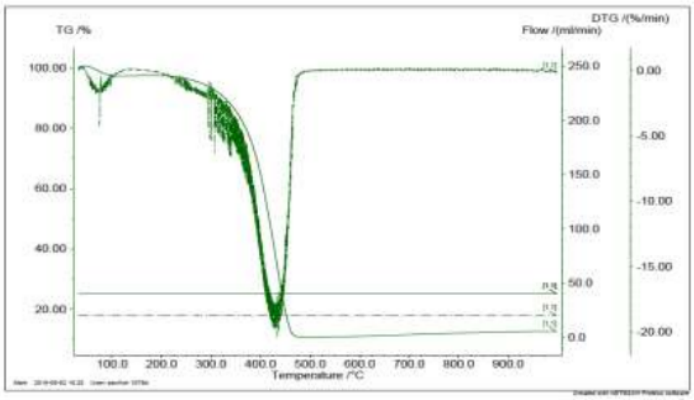


Figure 4. DTG thermogram of MIP_MAA-co-EGDMA$_{(AE)}$.

The DTG thermogram of MIP_MAA-co-EGDMA$_{(AE)}$ shows that there are several mass losses during heating of the material. The mass loss starts at a low temperature of 46.91 ^{o}C to 225.91 ^{o}C. The mass loss at this initial temperatures is suspected to be due to water loss at the temperature range. The great mass loss occurs at temperatures of 380.91 ^{o}C to 470.91 ^{o}C in which at this range, MIP is suspected to be degraded to CO and CO_2. At higher temperatures, bonds between monomers broken and only carbon remained. The high final temperature degradation indicates that MIP_MAA-co-EGDMA is stable.

3.2. The adsorption ability test

The amount of β-sitosterol adsorbed MIP_MAA-co-EGDMA and NIP_MAA_co-EGDMA was 0.72 and 0.06 mg/g, respectively. This shows that the adsorption ability of the former is better than that of the later. The same trend was also reported in the previous study [12]. The adsorption ability shown by the adsorption capacity values obtained from calculations using Langmuir and Freundlich isothermal *i.e.* 2.03 and 1.05 mg/g, correspondingly.

3.3. Selectivity test

The selectivity test was performed using the best carrier solvent composition. The solvent was mixed with β-sitosterol and cholesterol to carry both materials through a minicolumn containing MIP. The MIP can adsorb more β-sitosterol than cholesterol. The amount of β-sitosterol that can be adsorbed by MIP depends on the polarity properties of the solvent composition and the type of MIP. The polarity difference of the solvent was made from the composition ratio of methanol(M):water(W). The amount of β-sitosterol and cholesterol adsorbed was analyzed with HPLC to determine the percentage of effectiveness. The effectiveness percentage of β-sitosterol and cholesterol adsorption by MIP can be seen in table 2.

Tabel 2. The adsorption effectiveness of β-sitosterol dan cholesterol with several carrier solvent compositions.

MIP type	Carrier Solvent Composition	Adsorpstion Effectiveness Cholesterol (%)	Adsorpstion Effectiveness β-sitosterol (%)	Difference Adsorpstion Effectiveness (%)
MIP_MAA-co-EGDMA	M:W (90:10)	100	100	0
	M:W (80:20)	30.27	100	69.73

MIP_MAA-co-EGDMA can adsorb 100% cholesterol and β-sitosterol with the methanol:water composition of 90:10 (% v/v). When the polarity of solvent increases polarity such as in methanol ratio: water of 80: 20 (% v/v), MIP can adsorb 100 % β-sitosterol while cholesterol adsorbed is only 30.27 %. This because cholesterol is more polar than, whereas MIP_MAA-co-EGDMA is nonpolar. Based on these data, the carrier solvent composition used for MIP to be selective against β-sitosterol is the methanol:water ratio of 80:20.

The data of the effectiveness percentage proves that MIP_MAA-co-EGDMA has better selectivity against β-sitosterol than cholesterol. The effectiveness of a polymer is strongly influenced by the stability of the polymer. The mold is unchanged, therefore, the functional group in the polymer will only bind to the molecule whose functional group corresponds to the mold in the polymer. The MIP selectivity also relies greatly on the monomers and crosslinkers used [10]. Hashim *et al.* [13] reported that polymers made from MAA and EGDMA had high selectivity to stigmasterol used as their mold molecules, as in this study using MAA and EGDMA has a high selectivity for β-sitosterol. However, compared to MIP_TFMAA-co-EGDMA [14], MIP_MAA-co-EGDMA is more selective because the polymer adsorbed more cholesterol.

3.4. Adsorption-Desorption Test of MIP

The adsorption-desorption test of MIP was conducted in order to know the possibility of MIP to be reused. The recovery percentage of β-sitosterol from the used MIP_MAA-co-EGDMA in the second adsorption-desorption process reached 68.48%. Fauziah *et al.* [14] also tested the adsorption-desorption ability of MIP_TFMAA-co-EGDMA against β-sitosterol. The results obtained showed that the recovery percentage of β-sitosterol from MIP_MAA-co-EGDMA were better than that from MIP_TFMAA-co-EGDMA (35.56 %) for the second adsorption-desorption process.

3.5. MIP application

The MIP produced in this study was utilized as an adsorbent in a solid phase extraction (SPE) method in order to get information on the ability of the material to separate β-sitosterol from natural product samples and simultaneously can be used to facilitate the separation of other compounds from the β-sitosterol which is often the dominant compound in natural product samples. Table 3 shows the peak areas data of β-sitosterol obtained from chromatograms. The peak areas are used as data which are proportional to the amount of β-sitosterol present in the filtrate that has passed the SPE adsorbent. The greater the value of the area obtained, the greater the amount of β-sitosterol presents in the filtrate. The SPE method used for MIP applications as adsorbents was carried out through several important stages, *i.e.* retention, washing, and elution.

Table 3. Peak areas of β-sitosterol obtained from chromatograms at several stages in the SPE method.

Types	Stage on SPE method	Solvent	Peak area
Jackfruit		Methanol	775.6778
MIP_MAA-co-EGDMA	Retention	Jackfruit extract solution	28.7730
	Washing	methanol:water (8:2)	344.3110
	Elution	Methanol 100%	247.5000

4. Conclusion

Characterization of MIP_MAA-co-EGDMA using SEM, TGA and DTG showed that MIP_MAA-co-EGDMA was composed of highly regulated granules and resembled balls of similar size. The

structural geometry of polymers was more rigid and the polymers were stable to temperature. The functional groups that play a role in MAA synthesis and characterization were OH, C=O and C=C.

MIP_MAA-co-EGDMA had high adsorption ability (q_e) and the recovery percentage of β-sitosterol from MIP were 68.48 %. The MIP was very selective to β-sitosterol compared to cholesterol with the adsorption effectiveness to β-sitosterol of 100% and that to cholesterol of 30.27 %. The adsorption ability of MIP_MAA-co-EGDMA (0.72 mg/g) was better than that of NIP_MAA-co-EGDMA (0.06 mg/g).

Acknowledgement
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