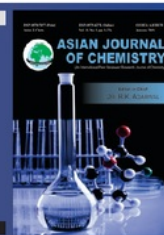


Adsorption Capacity and Selectivity of Molecularly Imprinted Polymers towards ?-Sitosterol

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Adsorption Capacity and Selectivity of Molecularly Imprinted Polymers towards β -Sitosterol

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Received: 21 April 2019;

Accepted: 11 June 2019;

Published online: 28 September 2019;

AJC-19581

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Molecularly imprinted polymers (MIP) as an adsorbent has been synthesized using β -sitosterol as molecule template on free radical polymerization reaction. The capacity and selectivity of the adsorption from MIP to β -sitosterol was studied in this study. The β -sitosterol concentration in the adsorption-desorption test and the MIP selectivity test were analyzed by UV-visible and HPLC. The MIP obtained from the synthesis results in a high adsorption capacity. Based on the Freundlich adsorption isothermal equation, the adsorption capacity (k) was found to be 1.24 mg/g. The MIP can adsorb 100 % β -sitosterol while cholesterol was only 3 %. The MIP is most selective to β -sitosterol, therefore, has high potential to apply as adsorbent at solid phase extraction method to isolate β -sitosterol from sample extract.

Keywords: Synthesis, Molecularly imprinted polymers, Selectivity, β -Sitosterol.

INTRODUCTION

The process of isolation and purification of a compound from natural product still has constraints for the researchers, due to the need of a lot of solvents, long process sequence and a high cost. Currently, a highly developed method is widely used for separation and purification process of compounds solid phase extraction. The method is superior compared to other conventional separation methods because of being more efficient and simple [1,2] but also has weakness if utilized in solid or adsorbent phases are not selective against target molecules [3].

Currently, there is known polymeric material called smart material. The type of polymer is an adsorbent with high selectivity and can know the target compound selectively [4]. These polymers are molecularly imprinted polymers (MIPs) synthesized using molecular imprinting techniques that require template molecules as cavity printers in polymers [2]. The template molecules can interact covalently and non-covalently with functional polymer-forming monomers. The flexibility of MIP formed from non-covalent interactions is preferred because the binding site is more heterogeneous so that the resulting MIP is more varied. The non-covalent interaction of binding is weaker so that it is easily decided compared to the covalent interaction that must be decided with an acid solvent [5,6].

The template molecules in polymer are re-extracted with organic solvent so that leaving a printed cavity corresponds to the size, shape and functional group position of molecule template [7]. The mechanism of interaction between the target compound and MIP are similar with enzyme working such as lock and key that occurs due to suitability of shape, size, and functional groups of the target molecule/s [8,9]. The characteristics cause MIP has a high selectivity and adsorption capacity to the target compound. In addition, MIP has high stability, can be stored for a long time at room temperature without losing its capacity effect and can be reused [10]. Molecularly imprinted polymer (MIP) has been widely applied to solid phase extraction, chromatography and sensors [11]. It is also made as a selective sorbent on solid phase extraction (SPE) to overcome the problem of separating certain analytes in a sample or pre-concentration of an analyte from a complex matrix [12], for example MIP is used to separate isomeric compounds, enantiomers from natural material samples [13], separation of colouring residues, contaminants of other chemicals such as melamine in food ingredients [14], separation of organic pollutants, herbicide residues contained in water and soil [15,16]. In sensors, MIP is used as a membrane (film) which can act as a signal transducer that converts binding signals from molecular recognition into detectable electrical signals [11], for example MIP

is used to detect certain compounds in drugs [17], human urine [18]. Meanwhile, MIP in chromatography is used as a stationary phase in the process of separating compounds in the sample. Chromatographic instruments such as HPLC, HPLC-MS and GC-MS are able to identify molecular structures adsorbed. In addition, the limit of detection and for the benefit of an effective enrichment approach can be reduced [11].

In this study, molecularly imprinted polymer (MIP) was synthesized and determined the adsorption capacity, selectivity and its application as adsorbent towards β -sitosterol using solid phase or chromatography extraction method.

EXPERIMENTAL

The chemical used in this study were 99 % methacrylic acid (Aldrich-Sigma), 97 % β -sitosterol (Aldrich-Sigma), trimethyl propane trimethacrylate 98% (Aldrich-Sigma), toluene, 2,2'-isobutyronitrile (Aldrich-Sigma), tetrahydrofuran (THF) and acetic acid (E. Merck). Sterile water and methanol of HPLC grade were purchased from Merck, USA. Equipment used includes glassware, shaker, digital balance, water bath, oven and thermometer, Agilent 1260 infinity HPLC instrument with Cronus RP E18C column type and column length 12.5 cm $\times$ 0.4 cm and Ultra violet-visible (UV-Vis) Spectrophotometer Agilent 8543.

Synthesis of MIP: A 2 mL of methacrylic acid (MAA) monomers and 0.05 g β -sitosterol were dissolved in a round flask containing 10 mL of toluene (9.41 mmol) as a pure solvent. Then, 3 mL of 7-methylolpropane trimethacrylate (TRIM), (9.3 mmol) as a cross-linker and 0.05 g (0.3 mmol) of azobisisobutyronitrile (AIBN) as initiator were added. Sonication was conducted to the solution and then flowed with nitrogen to remove dissolved oxygen. The polymerization process in water bath was carried out at 55 °C for 24 h. The polymer synthesized was dried and washed with tetrahydrofuran solvent, methanol/acetic acid (9:1) and sterile water to release the template, porogenous solvent and other compounds. The washed polymer was dried at 50 °C until dry. Non-imprinting polymers (NIPs) were made without template molecules with the same synthesis procedure such as MIP.

Adsorption ability of MIP: A total 3 mL of β -sitosterol solution with five of concentrations variation of 2, 4, 6, 8 and 10 ppm with the pH adjusted to suit the best pH incorporated into 5 vials containing 20 mg MIP. The mixture was stirred with a shaker for 60 min at the room temperature, then the mixture was filtered and β -sitosterol concentration in the filtrate were analyzed by a UV spectrophotometer at a wavelength of 202 nm. The adsorption capacity was determined using Langmuir and Freundlich adsorption isotherms.

Selectivity of MIP: The selectivity analysis was carried out by preparing a mini-column containing 50 mg of pre-saturated MIP with methanol. A 0.5 mL standard solution of cholesterol (10 ppm) was mixed with 0.5 mL standard solution of β -sitosterol (10 ppm), subsequently inserted into 2 mL carrier solvent containing some methanol and water composition. The solvent mixture is flowed into a mini column containing MIP. The solvent passed the columns was collected each 1 mL into 3 vials, then 100 % of methanol was flowed and also collected each other 1 mL into the 3 vials. Then all the solution in 6 vial

was analyzed by HPLC. The solution containing cholesterol and β -sitosterol were flowed using eluent composition of acetonitrile:methanol (80:20, v/v) with flow rate was 1 mL min⁻¹ at wavelength (λ) 205 nm.

The percentage of adsorption effectiveness and difference of adsorption effectiveness by MIP to β -sitosterol and cholesterol from HPLC analysis result was calculated to know the MIP selectivity to β -sitosterol. The amount of cholesterol and β -sitosterol results from HPLC analysis adsorbed on MIP was calculated using the equations:

$$\text{Adsorption effectiveness (\%)} = \left(\frac{C_o - C_e}{C_o} \right) \times 100$$

where C_o and C_e are the initial concentration and final concentration of cholesterol and β -sitosterol solution [4].

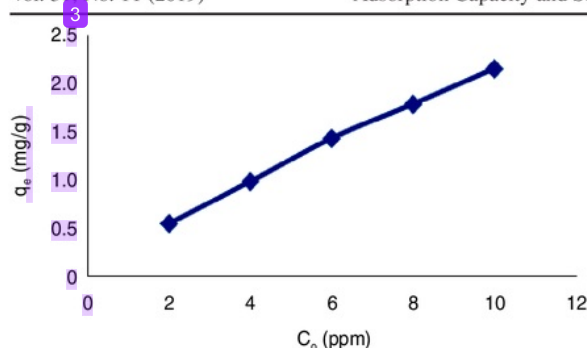
Adsorption-desorption analysis of MIP: The desorption-adsorption test was conducted by preparing a mini-column containing 50 mg of MIP saturated with methanol. A 0.5 mL standard solution of β -sitosterol (10 ppm) was inserted into 2 mL of solvent carrier containing with best ratio composition of methanol and water which have been used in selectivity test. The solvent mixture was flowed by peristaltic pump to a mini column containing 50 mg MIP in order to adsorption process to β -sitosterol. The solvent mixture passed the columns was collected each 1 mL twice. If the carrier solvent containing β -sitosterol is discharged then 100 % methanol is flowed to desorb again β -sitosterol from MIP. The solvent passed through the columns also collected each 1 mL twice. Then the results of elution collected were analyzed by UV spectrophotometer at the wavelength of 202 nm.

Application of MIP: A wood pulp plant powder (50 g) were dissolved in 100 mL of *n*-hexane solvent. The extraction was carried out at room temperature for 24 h. The extracted solution was filtered and concentrated using a rotary evaporator. Now jackfruit extract (10 mg) was dissolved into a 10 mL of methanol and filtered. Then, 1 mL of jackfruit extract solution was incorporated slowly into the SPE cartridge containing 100 mg MIP. The SPE cartridges containing MIPs were conditioned by passing methanol several times.

The β -sitosterol contained in the jackfruit extract solution is expected to retain on MIP as an adsorbent in SPE cartridge, and an unexpected component will be eluted. Solutions passed the MIP were collected and analyzed with HPLC. Then, the MIP adsorbed β -sitosterol was washed using 1 mL of the best solvent chosen from the selectivity test results in order to remove the other components from MIP. The solvent passed through the MIP in the cartridge was collected and analyzed by HPLC. The final step is extracting the retained β -sitosterol in MIP using 100 % methanol and 1 mL of solution from the SPE cartridge was collected and analyzed using HPLC.

RESULTS AND DISCUSSION

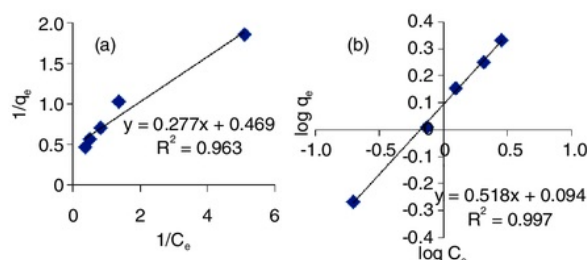
Adsorption capacity of MIP: Fig. 1 shows that an amount of β -sitosterol adsorbed (q_e) by MIP increases with the increasing initial concentration of standard β -sitosterol solution used. The equilibrium state of MIP has not been reached until the highest concentration used in this study, but if the concentration continues to increase then a particular time will be reached at

Fig. 1. Adsorption isothermal β -sitosterol on MIP_MAA-co-TRIM

the equilibrium state when the adsorption capacity of MIP will tend to be constant even though the concentration is continuously increased.

The adsorption capacity can be determined by using the Langmuir and Freundlich adsorption isotherms model. Fig. 2 shows the isotherms of Langmuir and Freundlich respectively for MIP_MAA-co-TRIM. The Langmuir isotherm curve was obtained by using the Langmuir isotherm equation:

$$1/q_e = (1/Q_0 b \times 1/C_e + 1/Q_0)$$

Fig. 2 (a) Langmuir isothermal curve and (b) Freundlich isothermal curve of β -sitosterol adsorption by MIP_MAA-co-TRIM

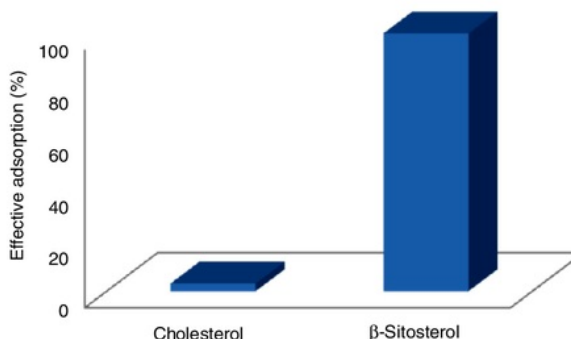
Linearity of the curve was obtained from the $1/q_e$ and $1/C_e$ relations, whereas Freundlich isothermal curve linearity was obtained from the $\log q_e$ relations and $\log C_e$ on Freundlich isothermal equation ($\log q_e = 1/n \log C_e + \log K$). The correlation coefficient value (R^2) obtained from the Langmuir adsorption isothermal curve for MIP_MAA-co-TRIM is 0.963; while the correlation coefficient value (R^2) obtained from the Freundlich adsorption isothermal curve for MIP_MAA-co-TRIM is 0.997. MIP_MAA-co-TRIM has a correlation coefficient value (R^2) for the Freundlich isotherm model larger than the Langmuir isotherm model so that the adsorption takes place on MIP corresponds to the Freundlich isotherm model.

Table-1 shows that the Freundlich (k), a constant value which describes the adsorption capacity of MIP_MAA-co-TRIM is greater than the MIP_MAA-co-EGDMA adsorption

capacity synthesized by Soekamto *et al.* [19]. This suggests that the MIP synthesized using the same monomer but using different cross linkers will provide different adsorption capabilities. This is in accordance with the results reported by Walsh [20]. MIP synthesized using a combination of MAA and TRIM has a better adsorption capacity than MIP synthesized using a combination of MAA and EGDMA because TRIM as a crosslinker has more vinyl groups. The vinyl functional group can contribute to binding with suitable monomer to result a stronger and more stable polymer.

A stable polymer can affect the adsorption capacity of the resulting MIP. Beltran *et al.* [21] reported that the stability of MIP will depend on the types of crosslinkers and the monomers used and MIP adsorption capacity to target molecule is strongly influenced by the stability of synthesized MIP.

Adsorption selectivity of MIP: The selectivity of MIP_MAA-co-TRIM to β -sitosterol can be determined by testing adsorption on β -sitosterol and cholesterol as a comparison because molecular structure cholesterol is similar to β -sitosterol. The selectivity analysis was conducted using a methanol and water composition (80:20, v/v) as carrier solvent. The mixing of carrier solvent and standard solution of β -sitosterol and cholesterol was flowed through a mini-column filled with MIP. Filtrate passed through MIP to be analyzed using HPLC to determine the adsorbed concentration. Amount of β -sitosterol and cholesterol adsorbed by MIP was expressed in effective percentage of adsorption. The difference of adsorption effective percentage of MIP to β -sitosterol and cholesterol was used to determine MIP selectivity. Comparison of effective adsorption percentage from MIP to β -sitosterol and cholesterol are shown in Fig. 3.

Fig. 3. Effective adsorption percentage by MIP_MAA-co-TRIM towards cholesterol and β -sitosterol

The effectiveness of MIP adsorption to β -sitosterol is 100 %, but the effectiveness of MIP adsorption to cholesterol is only 3 %. This proves that MIP is selective towards β -sitosterol. Selectivity of a MIP is also strongly influenced by the cross-

TABLE-1
ADSORPTION PARAMETER OF β -SITOSTEROL BY MIP_MAA-co-TRIM FROM
ADSORPTION ISOTHERMAL CURVE OF LANGMUIR AND FREUNDLICH

Type of MIP	Langmuir adsorption isothermal			Freundlich adsorption isothermal		
	Q_0	B	R^2	k	N	R^2
MIP_MAA-co-EGDMA	2.03	1.07	0.966	1.05	1.89	0.967
MIP_MAA-co-TRIM	2.13	1.69	0.963	1.24	1.93	0.997

TABLE-2
PEAK AREA DATA OF β -SITOSTEROL CHROMATOGRAM OBTAINED FROM SEVERAL STAGE ON COLUMN CHROMATOGRAPHY

Sample type	Step on SPE method	Solvent	Peak area
Jackfruit stem bark extract		Methanol	775.6778
MIP_MAA-co-TRIM	Retention	Jackfruit stem bark extract solution	135.84
	Washing	Methanol:water (8:2)	69.8978
	Elution	Methanol 100 %	598.802

linkers used. TRIM is a superior crosslinker in several applications because it contains many vinyl groups which contribute to binding in formation of a stable polymer matrix. The stability of the polymer causes the active side of polymer printing to be also stabilized because printing shape does not change. A stable printing will only bind molecules that is suitable with a printing shape. The MIP selectivity that was made using TRIM crosslinking was evident in this research and a stable polymer will have high selectivity [20]. A research that was conducted by Zhu *et al.* [22] also shows that polymers made from methyl methacrylate and TRIM have high selectivity to 17- β -estradiol as their printing molecules.

Adsorption-desorption of MIP: The MIP to be used repeatedly should be tested its adsorption desorption capabilities repeatedly. In this study, the adsorption and desorption tests was carried out twice and gave a high recovery percent value.

The results of β -sitosterol recovery percent of MIP for the first adsorption-desorption process obtained was 78.75 % value, whereas for the second adsorption desorption process was 71.9 % value. The value of β -sitosterol recovery percent by MIP for second usage is still very high and this indicates that MIP may be used more than once.

Application: The MIP was applied to column chromatography to prove its ability as a stationary phase as a substitution of silica gel at conventional chromatography for the separation of β -sitosterol from natural samples. Samples of natural ingredients used are jackfruit stem bark extract. The chromatogram peak area data of β -sitosterol are shown in Table-2. The peak area of β -sitosterol chromatogram obtained from HPLC analysis is considered to be directly proportional to the amount of β -sitosterol present in the filtrate. The greater the value of area of chromatogram then the amount of β -sitosterol present in the filtrate is also more. The MIP application process as an adsorbent on column chromatography will be conducted through several important stages namely retention, washing and elution stages.

In the retention step, the sample solution is passed to a column containing MIP to allow β -sitosterol to be retained by MIP. The low peak area indicates that β -sitosterol is well retained by MIP. The washing stage was carried out using a wash solvent to release the compound which is not retained by MIP. The peak area values obtained at the washing stage are very low. This suggests that β -sitosterol is well retained by MIP. The elution stage was conducted to release β -sitosterol retained on MIP with using 100 % methanol as the eluting solvent. The β -sitosterol retained on MIP-MAA-co-EGDMA can be eluted properly by 100 % methanol solvent and obtained a very high peak area.

Based on peak area data of HPLC chromatograms show that MIP application as an adsorbent on column chromatogra-

phy proved to be able to use instead of conventional stationary phase in convention column chromatography method to separate β -sitosterol from natural samples. It is also supported by the results of adsorption ability test and MIP-MAA-co-TRIM selectivity test against β -sitosterol is very good.

Conclusion

The MIP synthesized through the polymerization process using a bulk method consist of a white solid. Based on Freundlich isotherm adsorption equation, adsorption capacity value on MIP was found to be 1.24 mg/g. The percentage of recovered values for twice process of adsorption and desorption to MIP were 78.75 and 71.96 %. The MIP has a high selectivity to β -sitosterol with an adsorption effectiveness 100 % value to β -sitosterol, whereas the effectiveness value to cholesterol was only 3 %. The synthesized MIP can be applied to column chromatography as adsorbent to separate β -sitosterol from other compounds.

ACKNOWLEDGEMENTS

Thanks to the Directorate of Higher Education Indonesia for funding support. The authors also thanks to Hasanuddin University for facilitating the research activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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