Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Retnaningtyas & al. / Mor. J. Chem. 8 N°3 (2020) 588-593

Synthesis and Characterization of Molecularly Imprinted Polymer as Adsorbent for D-arabinitol

Yuni Retnaningtyas[1,2], Ganden Supriyanto[1,*], Ni Nyoman Tri Puspaningsih[1], Roedi Irawan[3]

[1]*Department of Chemistry, Faculty of Science and Technology, Airlangga University*
[2]*Faculty of Pharmacy, University of Jember*
[3]*Deparrment of Paediatrics, Faculty of Medicine, Airlangga University*

** Corresponding author:*
ganden-s@fst.unair.ac.id
yuniretnaningtyas@unej.ac.id
Received 28 April 2020,
Revised 5 May 2020,
Accepted 15 May 2020

Abstract

D-arabinitol is a metabolite product typically of several *Candida* species that are pathogenic and potentially used for candidiasis markers. In this study, a molecularly imprinted polymers (MIPs) of D-arabinitol has been synthesized and functions as D-arabinitol adsorbent. MIPs will functioned as an adsorbent after removing the template. The resulting polymers was characterized by FTIR, LC-MS and its binding efficiency tested with a batch binding assay. The results of FTIR analysis showed that the MIPs produced contained both monomers and templates. The template removal process was successful enough to see a significant reduction in the D-arabinitol area (no template detected) with LC-MS. The results of the batch binding assay indicated that MIPs has a binding capacity value (Q) of 1.075 mg/g whereas NIPs has a value (Q) of 0.131 mg/g. The results showed that the MIPs produced was potentially used as an adsorbent of D-arabinitol.

Keywords: *D-arabinitol; candidiasis; molecularly imprinted polymer; adsorbent*

1. Introduction

D-arabinitol is a pathogenic metabolite typical of several *Candida* species. The serum and urine levels of this product will increase if the involved fungus multiplies in the organism, causing invasive candidiasis [1,2]. In the late 1970s, high levels of the metabolite were found in the blood of patients with disseminated candidiasis. However, the use of it's as a diagnostic tool has low specificity, because high levels of D-arabinitol in blood not only found in patients with candidiasis but also in those suffering from kidney dysfunction. To avoid incorrect results due to kidney damage, its levels are usually expressed as ratios of D-arabinitol to creatinine or D to L-arabinitol [3,4]. D and L-arabinitol are isomers whose separation requires special technical, one of which being the use molecular imprinting polymers (MIPs). These are a techniques for producing a polymer with cavities due to the removal of a template, where the cavity recognizes molecules with the same size, structure and physical chemical properties as they are [5]. The purpose of this study is to synthesize the non covalentntly imprinted D-arabinitol polymers, characterized of the MIPs produced by FTIR and also evaluated the binding characteristics of MIPs to the template using batch ribinding assay [6].

2. Experimental

2.1. Materials

D-arabinitol, acrylamide (AA), ethylene glycol dimethacrylate (EGDMA), dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Co. Ltd. (United States), benzoyl peroxide (BP), methanol (MeOH), chloroform were obtained from Merck (Germany) and acetic acid (AcOH) was purchased from Alpha Chemika (India). All other chemicals and reagents were are the highest available purity and use as purchased.

2.2. Synthesis of MIPs and NIPs of D-arabinitol

The polymers were made by dissolving D-arabinitol as a template and AA as a functional monomer to DMSO as a porogen in a glass bottle. It then added with EGDMA as a cross-linker and added with BPO solution in chloroform as a initiator. The mixture was stirrrred for 10 minutes in order to allow complete dissolution. The resulting solution was purged with nitrogen to remove oxygen for 15 minutes. After that the glass bottle was closed and stored in water bath. Polymerization was carried out at 60ºC for 12 hours [7]. Non Imprinting Polymers (NIPs) as controls were synthesized in the same way, except for the addition of templates. The polymers formed (MIPs and NIPs) were then filtered out with a vacuum pump and put in an oven at 40℃ to dry and then crushed and sieved with 100 mesh sieves to produce a polymer with the size 50 mm or smaller.

2.3. Polymer Washing

Template removal was carried out by washing the polymer with a mixture of MeOH and AcA (1: 4) [8]. The process was carried out repeatedly until no more D-arabinitol could be detected in the washing liquid. Template analysis in the washing solution was carried out by LC-MS. The particles were then washed with methanol and water to remove residual acetic acid, followed by oven drying.

2.4. Batch Rebinding Assay

The efficiency of polymer binding to D-arabinitol was assessed through Batch Rebinding Assay. MIPs and NIPs of 50 mg each were placed in a vial containing 10 mL, 8 µg mL^{-1} D-arabinitol solution in methanol (defined as early D-arabinitol) (Co) then stirred mechanically at room temperature. The samples were taken at different time intervals (1, 2, 3, 4, 5, 6 and 7 hours). After that, the polymer was centrifuged at 5000 rpm for 10 minutes to separate the liquid

from the solids of the remaining substrate in solution (defined as free D-arabinitol) (Ci) analyzed through LC-MS to permit the measuring of the amount of D-arabinitol remaining. NIPs was used as controls to determine non-specific bonds. The amount of D-arabinitol bound to the polymer is calculated by reducing Co with Ci. The binding capacity of MIPs and NIPs to D-arabinitol is calculated using the following equation:

$$\text{Binding capacity (Q)} = \frac{V(Co - Ci)}{W}$$

Where V is the volume of D-arabinitol solution added, Co is the early D-arabinitol concentration, Ci is the free D-arabinitol concentration after incubation, W is the mass of MIPs used [9].

3. Results and discussion

In this research, D-arabinitol MIPs and NIPs were prepared by bulk polymerization. Bulk polymerization is the general and easiest method of polymerization, although the obtained polymer needs to be crushed, ground and sieved, which is a laborious and time-consuming procedure [10,11]. AA was selected as functional monomers because the amide group of AA is a stronger hydrogen bonding functional group and it is also important to note that by using AA instead of basic or acidic monomers, polymers could be made without the existence of charged groups and thus the non-specific, background ionic interactions could be reduced [7]. EGDMA and BP were chosen as cross-linker and initiator. In our previous work, we practically found that the best D-arabinitol : AA : EGDMA ratio is 1 : 4 : 20, and the best polymerization solvent is DMSO. The polymer particles are obtained in the form of a white hard polymer block with a brittle structure. The crushed bulk polymers usually provide irregular shaped particles. In this study, bulk materials were crushed and sieved for obtaining particles with dimensions <50 mm. NIP was made in the same way but without the addition of the templates [11].

3.1. FTIR Characterization

AA used as a monomer s an amide group with a typical and dominant NH absorption around wave number 3400 cm^{-1}. The presence of peaks in the wave number region 3407.51 cm^{-1} and 1721.50 cm^{-1} in spectra FTIR MIPs shows a vibration carbonyl group C = O stretching from AA. Besides, the template D-arabinitol in the polymer produced is characterized by the presence of a weak peak in the wave number area 2955.75 cm^{-1}, showing the presence of C-H stretching vibrations from the template. The vibration in the wave number region 1388.09; 714.01; 641.41; and 617.01 cm^{-1} shows the presence of OH bending of D-arabinitol [12]. The FTIR spectrum of MIPs as shown in Figure 1 implies the resulting MIPs are a mixture of the amine, carbonyl and hydroxyl groups, and as expected. However, the FTIR NIPs spectrum as shown in Figure 1 shows in the resulting polymer there was no template D-arabinitol because there was no peak in the wave number region 2955.75 or 1388.09 cm^{-1}; 714.01; 641.41; 617.01 cm^{-1}, a marker group of D-arabinitol [13].

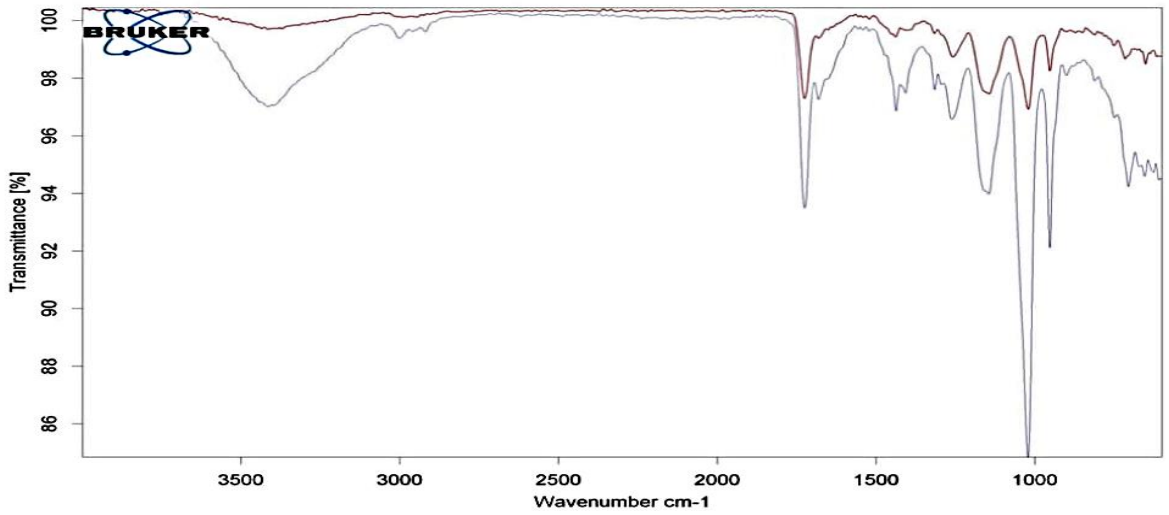
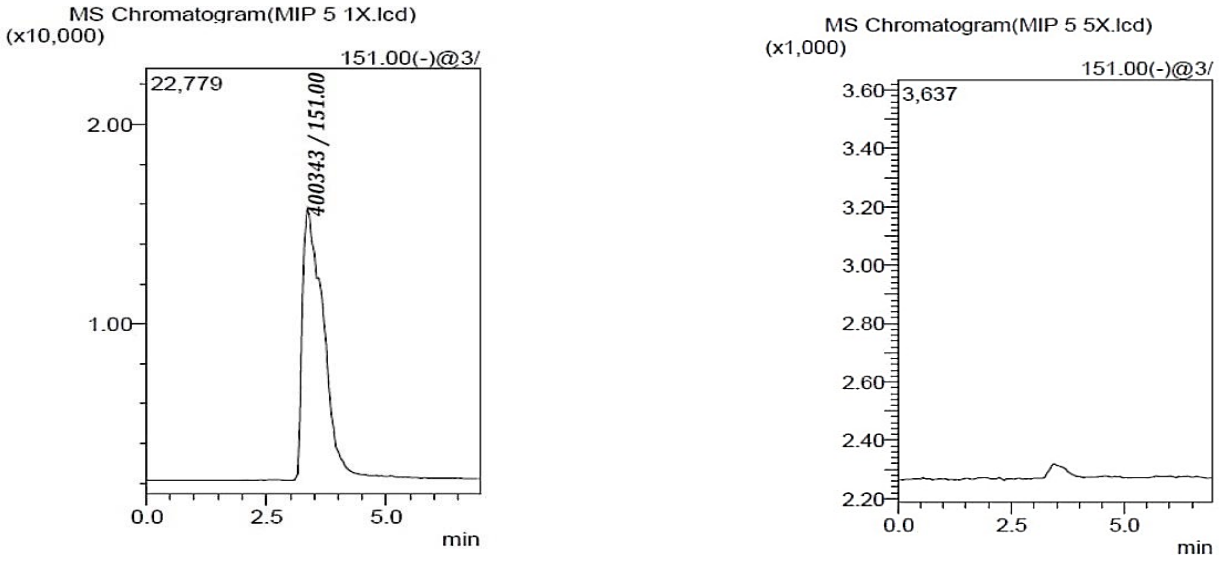


Figure 1. Spektra FTIR of MIPs (-) and NIPs (-)

As supporting data in determining the presence of D-arabinitol in polymers, LC-MS was used by washing MIPs. The results showed that the polymer contained D-arabinitol as presented on Figure 2a, indicated by it's detection in the washing liquid analyzed by LC-MS. After washing five times no peak was detected as presented on Figure 2b, it was indicated that the washing solution does not contain D-arabinitol, which means the whole of it was released from the polymer. The template removal of polymers is an important factor in determining the performance of MIPs because the more templates released, the more cavities produced. The number of cavities produced will determine how many analytes bound by the polymers.



(a) (b)

Figure 2. (a) Chromatogram liquid washing after the washing of one time; (b) Chromatogram liquid washing after the washing of five times

3.2. Batch Binding Assay of MIPs and NIPs

This study aims to determine the efficiency of binding of MIPs and NIPs to D-arabinitol. The binding efficiency of MIPs toward the templates in this study was carried out at optimum conditions. This is proven by the results of the

optimization that had been done previously and measured by LC-MS. The results of measuring binding efficiency at different time intervals for MIPs and NIPs against D-arabinitol are shown in Table 1 and Figure 3.

Table 1: Binding Capacity Value of MIPs dan NIPs

MIPs				**NIPs**			
Incubation Time (minutes)	Bound mass (mg)	MIPs mass (gram)	Binding capacity (Q)	Incubation time (minutes)	Bound mass (mg)	NIPs mass (gram)	Binding capacity (Q)
60	0.02	0.05	0.320	60	0.007	0.05	0.138
120	0.02	0.05	0.463	120	0.003	0.05	0.069
180	0.03	0.05	0.652	180	0.003	0.05	0.066
240	0.04	0.05	0.709	240	0.005	0.05	0.098
300	0.04	0.05	0.881	300	0.001	0.05	0.024
360	0.05	0.05	1.075	360	0.007	0.05	0.131
420	0.05	0.05	1.065	420	0.004	0.05	0.085

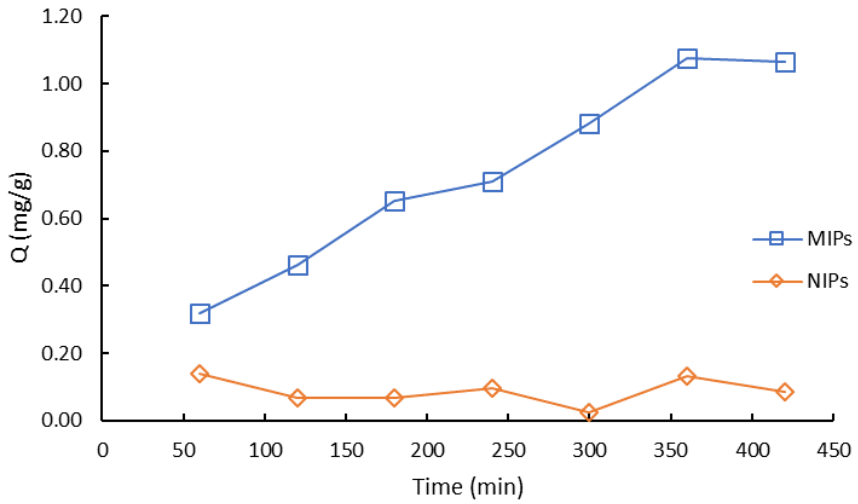


Figure 3. The binding capacity of MIPs and NIPs at different time intervals

Based on the data in Table 1 and Figure 3, it shows that D-arabinitol MIPs has a fairly large binding capacity (Q) (1.075mg/g) when compared with the Q value of NIPs (0.131mg/g). These results indicate that the synthesized MIPs have cavities with size, structure and physical chemical properties similar to D-arabinitol. This allow them to function as D-arabinitol identifiers. On the other hand, NIPs does not contain any binding site with the D-arabinitol.

4. Conclusion

In this study, the synthesis of D-arabinitol MIPs was successfully carried out through a non-covalent approach. FTIR spectra of MIPs D-arabinitol and analysis results of washing liquid with LC-MS showed that the polymer produced contained functional monomers and templates. Washing templates also managed to eliminate all templates from MIPs. MIPs produced have a much greater binding capacity value (1.075 mg/g) compared to NIPs (0.131mg/g). This result indicate that the MIPs were potentially be used as D-arabinitol adsorbent.

Acknowledgement

This works has been supported by Indonesian Government through the Indonesia Endowment Fund for Education (LPDP)

References

[1] W. Shaw, E. Kassen, and E. Chaves, "Increased urinary excretion of analogs of Krebs cycle metabolites and arabinose in two brothers with autistic features," *Clin. Chem.* 41 (1995) 1094–1104

[2] J. Kałuzna-Czaplińska and S. Błaszczyk, "The level of arabinitol in autistic children after probiotic therapy," *Nutr.* 28 (2012) 124–126

[3] A. N. Ellepola and C. J. Morrison, "Laboratory diagnosis of invasive candidiasis," *J. Microbiol.* 43 (2005) 65–84

[4] T. J. Stradomska, D. Sobielarska, Z. Mielniczuk, D. Jagiełłowicz, M. Syczewska, and D. Dzierzanowska, "Determination of urinary D-/L-arabinitol ratios as a biomarker for invasive candidiasis in children with cardiac diseases," *J. Med. Microbiol.* 59 (2010) 1490–1496

[5] B. Gao, Y. Chen, and J. Men, "Constructing chiral caves and efficiently separating enantiomers of glutamic acid with novel surface-imprinting technique," *J. Chromatogr. A* 1218 (2011) 5441–5448

[6] G. Vasapollo, R. D. Sole, L. Mergola, M. R. Lazzoi, A. Scardino, S. Scorrano, and G. Mele, "Molecularly Imprinted Polymers: Present and Future Prospective," *Int. J. Mol. Sci.* 12 (2011) 5908–5945

[7] B. Okutucu, S. Önal, and A. Telefoncu, "Noncovalently galactose imprinted polymer for the recognition of different saccharides," *Talanta* 78 (2009) 1190–1193

[8] R. Lahsini, M. R. Louhaichi, N. Adhoum, and L. Monser, "Preparation and application of a molecularly imprinted polymer for determination of glibenclamide residues," *Acta Pharm.* 63 (2013) 265–275

[9] Y. Suwanwong, S. Kulkeratiyut, V. Prachayasittikul, and S. Boonpangrak, "Effects of polymerization methods and functional monomers on curcumin imprinted polymer properties," *Sep. Sci. Technol.* 49 (2014) 1086–1095

[10] F. Schneider, S. Piletsky, E. Piletska, A. Guerreiro, and M. Ulbricht, "Comparison of thin-layer and bulk MIPs synthesized by photoinitiated *in situ* crosslinking polymerization from the same reaction mixtures," *J. Appl. Polym. Sci.* 98 (2005) 362–372

[11] S. A. Mohajeri, G. Karimi, J. Aghamohammadian, and M. R. Khansari, "Clozapine recognition via molecularly imprinted polymers; bulk polymerization versus precipitation method," *J. Appl. Polym. Sci.* 121 (2011) 3590–3595

[12] J. Coates, "Interpretation of Infrared Spectra, A Practical Approach," *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation* (2000) 10815–10837

[13] G. Li and K. H. Row, "Recent applications of molecularly imprinted polymers (MIPs) on micro-extraction techniques," *Sep. Purif. Rev.* 47 (2018) 1–18