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Synthesis and Characterization of Molecularly Imprinted Polymer with Dimethylamylamine as Template

S. AMIN[1,2,*], S. DAMAYANTI[1] and S. IBRAHIM[1,*]

[1]Bandung Institute of Technology, Jl. Ganesha No. 10 Bandung, Indonesia
[2]STIKes Bakti Tunas Husada Jl. Cilolohan 36 Tasikmalaya, Indonesia

*Corresponding authors: E-mail: saeful_amin@stikes-bth.ac.id; sibrahim@fa.itb.ac.id

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Molecular imprinted polymer (MIP) is a selective polymer that has the ability to bind target molecules so that they can be used as sorbents in the process of separation. Molecular imprinted polymer is made with a ratio of 1:4:20 between dimethylamylamine, methacrylic acid, and ethylene glycol dimethacrylate. The synthesis of molecular imprinted polymer was performed using bulk method with direct heating at 60 °C for 7 h. By using Fourier transform infrared, there is a functional group difference between the FTIR spectrum of molecular imprinted polymer, non-imprinted polymer and molecular imprinted polymer without dimethylamylamine. Scanning electron microscopy (SEM) shows that molecular imprinted polymer has an irregular and hollow morphological structure, unlike non-imprinted polymer that tends to be irregular compared to molecular imprinted polymer and have no cavities. While molecular imprinted polymer without dimethylamylamine has irregular and hollow morphological structure of equal size more than molecular imprinted polymer. The resulting molecular imprinted polymer has a selectivity to adsorb dimethylamylamine 209 % higher than non-imprinted polymer. The percentage of concentration of molecular imprinted polymer with chloroform solvent was 203 % more dissected than the percentage of molecular imprinted polymer with methanol solvent.

Keywords: Molecular imprinted polymer, Dimethylamylamine, Methacrylic acid, Non-imprinted polymer, Isotherm Adsorption.

INTRODUCTION

Exercise is the place of interaction among humans which contains ethical values between one another that must be shown, tested and studied. Sports contain lessons such as honest play, teamwork, sportsmanship and so on. The heavy burden of being a winner on the shoulders of an athlete can be fatal to him. Some athletes use various means which are prohibited in competition to achieve the desired target, one of them is through the use of doping [1].

Types of doping are diverse, but some of them are drugs that are prohibited to use by governments either in sports activities or outside sports activities [1]. One such example is 1,3-dimethylamylamine, which is listed as a doped agent and prohibited by World Anti-Doping Agency (WADA) in 2010 and belongs to non-specific categories of stimulants. While in "The 2017 Prohibited List International Standard", methacrylic acid is categorized into a category-specific stimulant class [2]. Currently, dimethylamylamine is widely used as a popular stimulant in pre-exercise, weight loss and improving the performance of dietary supplements [3].

Adverse cases due to the use of dimethylamylamine in supplements are common in sports activities as well as outside sports activities. In Anti-Doping Testing data 2015, the world class athletes used dimethylamylamine as a doping, out of which 11 % (56 cases) were recorded [4,5]. One of the adverse cases due to dimethylamylamine outside sport activity is the death of soldiers who are still actively serving in the US military after taking a dietary supplement containing dimethylamylamine [6].

Dimethylamylamine contained in dietary supplements is used to improve the performance that usually consist of various ingredients, especially containing proteins. Various methods have been developed for the separation of dimethylamylamine compounds, one of which is reported by Avila and Zorio [7] using GC-high resolution TOF mass spectrometry with soft ionization method. These developed methods require complex procedures and expensive analysis instruments resulting from

heterogeneous sample matrices. It is necessary to develop a separation method with high selectivity to analyte [8].

The separation methods with the adsorbent that have been developed are molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP). Molecularly imprinted polymer is a selective polymer that has the ability to bind target molecules to be used as sorbents in the separation process [9]. Osman *et al*. [10] succeeded in making caffeine MIP which was applied with acetic acid as an extractant template. The synthesis of MIP for the analysis of some compounds with methacrylic acid as monomers using several methods has also been done [11]. The difference between MIP and NIP lies in its synthesis composition where NIP is synthesized without template molecules and used as a comparison of MIP results. The use of MIP in extraction has high benefits because it provides selective extraction; easy and inexpensive sample preparation [12].

Based on the background above, MIP and NIP synthesis have used dimethylamylamine imprinted molecule and methacrylic acid monomer with 1:4 ratio using direct heating method. The synthesis of MIP and NIP and characterization using Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM) to determine the functional groups and their morphology, as well as determination of MIP and NIP. Synthesized adsorption and desorption capacity were examined using HPLC.

EXPERIMENTAL

Equipment used are Fourier transform Infrared (Jasco FTIR 4200), scanning electron microscope (JEOL JSM-6510), UV-visible spectrophotometer (Genesys 10), high performance liquid chromatography (Agilent Technologies 1120 Compact LC), oven (Memmert), analytical balance (Melter Toledo), sonicator, vortex, reagent bottle, funnel, micropipette, spatula, mesh 60 sieve and glass tools commonly used in pharmaceutical laboratory analysis.

The chemicals *viz*. dimethylamylamine, methacrylic acid, ethylene glycol dimethacrylate, azobisisobutyronitrile were procured from Sigma-Aldrich. Chloroform, ethyl acetate and methanol p.a. were purchased from Merck, which acetonitrile for HPLC and aquabidest procured from other sources.

Synthesis of polymer: The synthesis of MIP uses a bulk method made with a 1:4:20 ratio (in moles) between template molecule, functional monomers and crosslinking. Dimethylamylamine was dissolved in reagent bottle with chloroform and sonicated to ensure that dimethylamylamine was completely dissolved. Methacrylic acid was added to the reagent bottle and then reconstituted for 20 min for the introduction of template molecules to monomers. Ethylene glycol dimethacrylate (EGDMA) as a crosslinker, was added to reagent bottle and sonicated and then azobisisobutyronitrile (AIBN) as an initiator was added and sonicated again. To remove the oxygen trapped in the reagent bottle, the reagent bottle was replaced with nitrogen gas and tightly closed. The mixture in reagent bottle is heated for 7 h using the oven at 60 ºC. The formed crystals were observed every half hour. Then the polymer formed was sieved using 60 mesh. As a comparison, NIP was synthesized with the same composition and method of MIP, but without the use of dimethylamylamine as template molecule.

Releasing of template molecule: The template molecule (dimethylamylamine) release was performed on the synthesized MIP by extraction using chloroform and sonicated for 30 min. A total of 100 mg MIP was extracted using 10 mL of chloroform. The filtrate was collected for analysis using HPLC and the residue was dried using an oven at 45 ºC and analyzed. The release of template molecule is said to succeed if no more imprinted molecules are still present in the observed MIP of infrared spectra of MIP and NIP. When all the template molecule is released, the MIP will have the same spectra as NIP.

Characterization of polymers using FTIR: MIP before the extraction, after extraction MIP and NIP before extraction, were characterized by using FTIR. Preparation of the sample was done by using KBr pellet method in which powdered samples were mixed with KBr with a ratio of 1:9 and crushed to be homogeneous. The imprinted disc was then inserted and compressed at 20 psi pressure using a hydraulic press. Discs were mounted on the holder and then the spectrum was measured at wave numbers of 4000-400 cm^{-1} using FTIR spectrophotometer.

Characterization of polymers using SEM: SEM was done by means of sample attached to specimen holder then cleaned by hand blower. The cleaned sample was given a coating with gold palladium then inserted into chamber specimen and analyzed at 10,000x and 25,000x magnification.

Calibration curves of dimethylamylamine: Dimethylamylamine dilution sequence in chloroform with concentrations of 100, 200, 300, 400, 500 and 600 ppm was prepared by pipe from 1000 ppm dimethylamylamine storage solution into a 20 mL measuring flask, then chloroform was added to the homogeneous border mark. Read its AUC using HPLC, then make a calibration curve between concentration (ppm) and AUC. The equation of the line is determined by the linear regression equation.

Polymer adsorption capacity: Prepared dimethylamylamine dissolved in chloroform with various concentrations of 100, 200, 300, 400, 500 and 600 ppm. A 50 mg of NIP and MIP extracted, then incorporated into each dimethylamylamine solution. It was incubated for 24 h at room temperature and then filtered, the supernatant was measured using HPLC in order to observe the concentration of dimethylamylamine left in the solution. An adsorption curve was created between the adsorbed amount and the initial dimethylamylamine concentration. Imprinting factor (IF) is calculated using the following equation:

$$\text{Imprinting factor (IF)} = \frac{AUC_{MIP}}{AUC_{NIP}}$$

Polymer desorption capacity of various solvents: From MIP and NIP adsorption capacity results, each was added with 5 mL of chloroform solvent, ethyl acetate, methanol and then it was sonicated for 25 min. The mixture was filtered and measured by AUC filtrate at dimethylamylamine wavelength. A curve was created between the number of dimethylamylamine released every 10 mg of polymer to the concentration of dimethylamylamine used.

RESULTS AND DISCUSSION

Synthesis of polymers: The MIP and NIP syntheses is a type of free radical polymerization, while the method used is bulk

method. This method is adopted because it is fast, simple and does not require any special skills or advanced instruments in its synthesis. Consisting of imprinted molecules, functional monomers, crosslinkers, initiators and porogen solvents were initiated to form polymerization with the ideal composition of the ratio based on the optimization results of the literature study between imprinted molecules, functional monomers, and crosslinking for MIP synthesis which is 1:4:20 (in moles). The comparison is sufficient to produce a stable complex interaction [13].

Methacrylic acid is a monomer that can act as both a donor and an acceptor whose hydrogen bond forms a strong bond with the selected solvent, thereby increasing capacity and homogeneity of the cavity formed [14]. Ethylene glycol dimethacrylate (EGDMA) is used as a crosslinker due to its good porosity and easily polymerized [3]. Chloroform is used as a sporogenous solvent because chloroform is a solvent with a not so-high polarity. The excessively high solvent polarity cannot produce a selective MIP because of possibility of competition between the molecular interaction of imprinted and functional monomer, which will interact more with the porogen solvent. Polar sporogenous solvents will produce strong interactions with imprinted molecules and functional monomers causing interaction between imprinted molecules and functional monomers getting weaker [15].

The synthesized MIP dissolves dimethylamylamine in chloroform in the reagent and synchronized bottles to make sure its complete dissolution. Methacrylic acid is added to the reagent in synthetic bottles for the introduction of template molecule to functional monomers.Ethylene glycol dimethacrylate (EGDMA) is added to the reagent and synchronized. AIBN is added and sonicated to be entirely homogeneous. The reagent bottle is filled with nitrogen gas to remove oxygen trapped in the reagent bottle. The removal of oxygen gas by passing inert gas such as nitrogen needs to be done after the addition of AIBN because oxygen can slow down the polymerization by forming peroxides produced from the reaction between oxygen and free radicals formed [16]. Heating is performed for 7 h using an oven at 60 ºC. NIP is synthesized with the same number and conditions as MIP synthesis but without the addition of dimethylamylamine. MIP and NIP are organoleptically synthesized in white solids.

Releasing of template molecules: The release of template molecule aims to remove dimethylamylamine from MIP polymer matrix so that a three-dimensional cavity which can be formed re-bind dimethylamylamine. The release of imprinted molecular was performed using sonication method with chloroform as a solvent. The method of sonication is chosen because it is simple, it does not cause adverse effects on the morphology of the polymer and it gives good molecular release results. The release of template molecule is said to succeed if no more dimethylamylamine is still present in the observed MIP of infrared spectrum of MIP and NIP. When all dimethylamylamine is released then MIP will have the same spectrum as NIP.

FTIR studies: From the infrared spectra of dimethylamylamine, MIP and NIP (Fig. 1), absorption equation between MIP and dimethylamylamine which is not present in NIP can be seen in the functional group area. The spectrum present in N-H functional group region at 3301 cm^{-1} and in C-N fingerprint region at 1153 cm^{-1} in the form of a doublet due to the interaction of hydrogen bond between the imprinted molecule and monomer. This shows that dimethylamylamine is already polymerized into functional monomers and MIP synthesis is said to be successful.

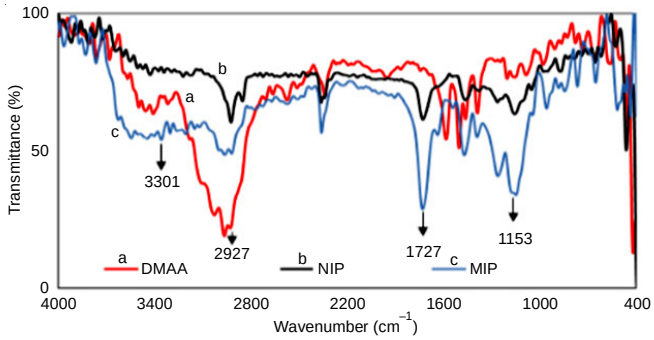

Fig. 1. FTIR spectra of dimethylamylamine, MIP and NIP

The methacrylic acid used as a monomer is a carboxylic acid group having a typical and predominant C=O uptake at 1820-1660 cm^{-1} [17,18]. In MIP, NIP and MIP without dimethylamylamine (Fig. 2) C=O, absorption is present at 1727 cm^{-1}. Fig. 2 shows that the infrared spectrum results in MIP without dimethylamylamine have been similar to the infrared spectrum on NIP both in the fingerprint area and the functional group region.

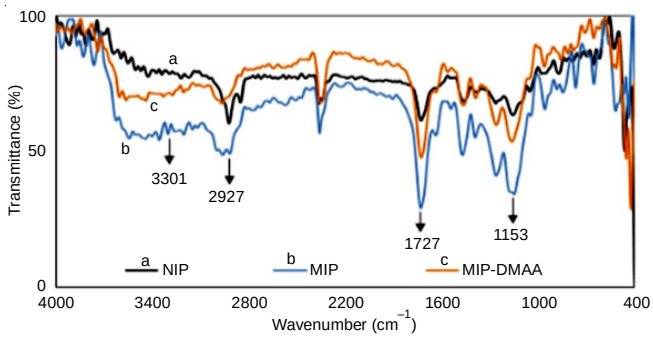

Fig. 2. FTIR spectra of MIP, MIP without dimethylamylamine and NIP

In MIP prior to release of template molecule, N-H group at 3301 cm^{-1} and in MIP after the release of molecular prints observed at 3328 cm^{-1}. The presence of a peak that shifts in the FTIR spectra towards a larger one after the release of template molecule shows the presence of hydrogen bonding interactions between the template molecule and monomer. The hydrogen bond interaction causes a decrease in electron density in the interacting group resulting in a decrease in vibration frequency [19].

SEM analysis: Based on Fig. 3, the morphological differences between MIP, NIP and MIP without dimethylamylamine. MIP has an irregular and hollow morphological structure is in contrast to NIPs that tend to be irregular compared to MIP and have no cavities. While MIP without dimethylamylamine has an irregular and hollow morphological structure of the same size more than MIP. The cavity formed in MIP without dimethylamylamine serves for the adsorption of molecules that have the same structure and size as those cavities. The regular NIP structure shows that no specific binding sites are formed by dimethylamylamine template molecule, whereas the cavities formed in

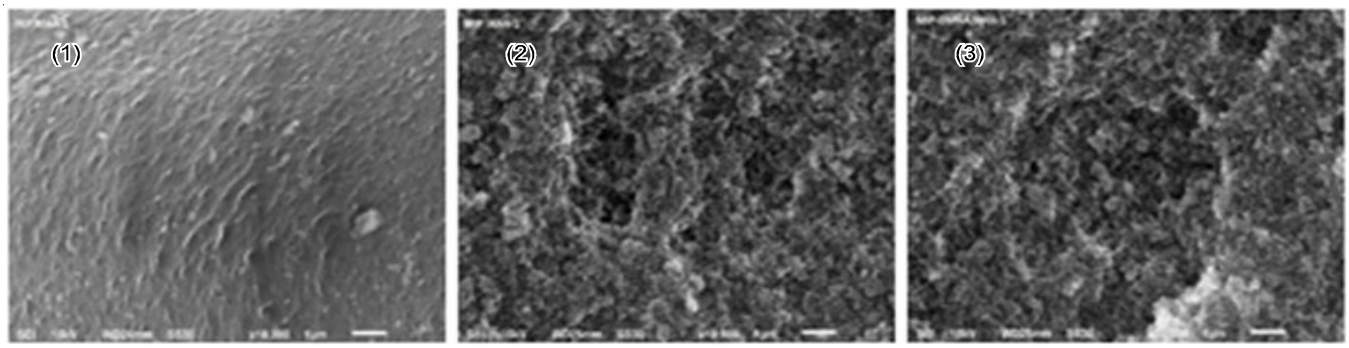

Fig. 3. Results of SEM characterization 10,000x: 1. NIP; 2. MIP; 3. MIP without dimethylamylamine

MIP are due to dimethylamylamine template molecule interacting with methacrylic acid polymers while NIP has no dimethylamylamine interaction with methacrylic acid polymers [20].

HPLC analysis

Scanning length of dimethylamylamine waves: Determination of dimethylamylamine wavelength is done by dissolving chloroform and its maximum wavelength at 200-800 nm measured using UV-Vis spectrophotometer with chloroform blank. The dimethylamylamine wavelength is obtained at 248 nm (Fig. 4).

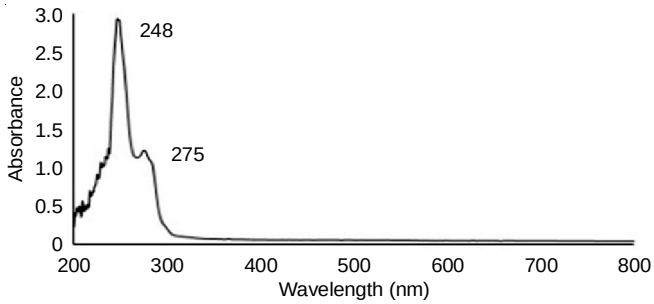

Fig. 4. UV-visible spectrum of dimethylamylamine

Calibration curve of dimethylamylamine: The calibration curve between concentrations with standard AUC dimethylamylamine in chloroform with concentrations of 100-500 ppm can be seen in Fig. 5.

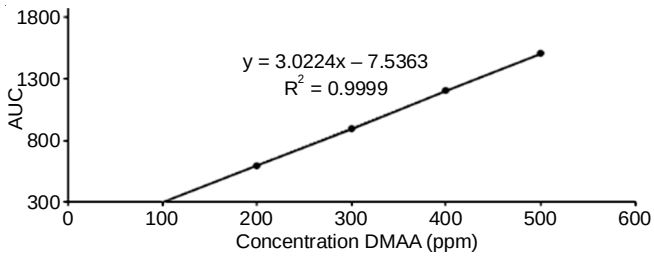

Fig. 5. Calibration curve of dimethylamylamine

The linear regression equation obtained from the result of calibration curve is y = 3.0224x + 7.5363 and regression coefficient (R^2) is equal to 0.999. The resulting regression coefficient (R^2) states that there are a close correlation and good linearity between the concentration of dimethylamylamine in chloroform with its AUC, since R^2 value range is in the range of $0.9 < R^2 < 1$ [21].

Adsorption capacity: The solvent used for determining the adsorption capacity is chloroform. The same solvent is used when polymerization is performed for the binding of imprinted molecules [13]. The adsorption capacity is formed by a batch method by sonication for 25 min for dimethylamylamine introduction of MIP cavity and incubated for 24 h to allow for dimethylamylamine interaction with MIP and NIP. After incubation, the solution is filtered and each of the obtained filtrates measures AUC at 248 nm.

The result of adsorption capacity determination in Fig. 6 shows that the MIP has higher selectivity to bind dimethylamylamine than NIP. The imprinting factor (IF) value in Table-1 is obtained by 2.09 indicates that MIP adsorges 209 % dimethylamylamine over NIP. Therefore, MIP has been made to be able to bind back dimethylamylamine higher than NIP. Isotherm can be matched using various models with different assumptions, namely Langmuir and Freundlich isotherm models.

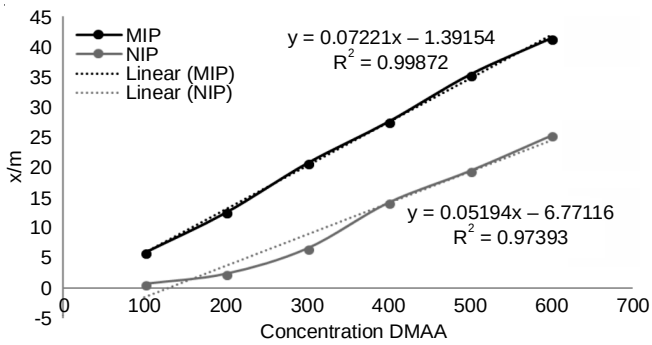

Fig. 6. Adsorption capacity curve

Based on the value of R^2 in Table-2, the NIP isotherm adsorption model follows the Freundlich isotherm model [22]. This model is based on the assumption that the sorbent has a heterogeneous surface and that each molecule of the template molecule has different absorption potentials [23]. However, after the addition of dimethylamylamine, in MIP, the isotherm adsorption model changes following the Langmuir isotherm model. This model explains the adsorption of template molecule to occur only on the surface of monolayer and the adsorption rate does not depend on the number of adsorbed molecules [24]. Based on the assumption, each side of adsorption has the same affinity for the template molecule. Adsorption on one side does not affect the other side [8]. Based on the value of ΔG in Table-2, the isotherm adsorption model follows the Langmuir isotherm model with the energy generated at MIP of -19.167 (J/mol) and at NIP of -15.405 (J/mol). The resulting negative energy means that the adsorption is spontaneous [23].

TABLE-2
LANGMUIR AND FREUNDLICH EQUATIONS

Polymer	Langmuir				Freundlich			
	R^2	A	B	ΔG (J/mol)	R^2	n	K	ΔG (J/mol)
MIP	0.999	0.056	-4.10^{-4}	-19.167	0.999	1.103	0.037	8.182
NIP	0.978	0.006	-0.002	-15.405	0.993	2.102	-4.10^{-5}	25.064

TABLE-1
VALUE OF IMPRINTING FACTOR
(IF) ON ADSORPTION CURVE

AUC MIP	AUC NIP	IF
11940.98	5703.92	2.09

Desorption capacity: MIP and NIP solids resulting from adsorption capacity can be used for desorption capacity determination. The solvent used to determine the adsorption capacity is not as strong or as polynomial as porogenic solvent. If the poles are stronger, there will be no dimethylamylamine displacement from the solvent to the binding side hole on MIP [13].

The desorption capacity in this study is carried out using some solvents from the lowest polarity to the highest polarity solvent *i.e.* chloroform, ethyl acetate and methanol. Reprocessing produces the best results, if the solvent used is the same solvent during polymerization [13]. According to Fig. 7, in MIP with a chloroform solvent, the percentage of concentration resorbed was 203 % more than the percentage of MIP with methanol solvent.The percentage of dissipated concentration between MIP and NIP using chloroform solvent was 115.4 %, methanol 114.7 % and ethyl acetate 0 %. The highest desorption concentration is in chloroform, which may be attributed due to the reason that chloroform is a porogenic solvent used during MIP and NIP synthesis.

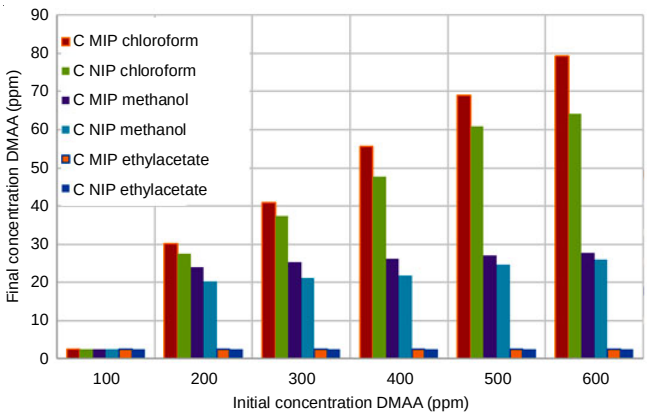

Fig. 7. Desorption capacity curve

Conclusion

Based on the research that has been conducted, MIP and NIP use dimethylamylamine as template molecule, methacrylic acid as monomer and EGDMA as a crosslinker with a ratio of 1:4:20 which can be synthesized using bulk method with direct heating. Characterization of MIP and NIP using FTIR indicates that there are different functional groups between MIP, NIP and MIP without dimethylamylamine. The SEM results shows the morphological differences between MIP, NIP and MIP without dimethylamylamine. The resulting MIP has a selectivity to adsorb dimethylamylamine 209 % higher than NIP has. The percentage of concentration of MIP with chloroform is 203 % more dissected than the percentage of MIP with methanol solvent.

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CONFLICT OF INTEREST

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

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