Medical Sains : Jurnal Ilmiah Kefarmasian
Vol. 9 No. 1, January - March 2024
https://ojs.stfmuhammadiyahcirebon.ac.id/index.php/iojs

SYNTHESIS AND CHARACTERIZATION OF MOLECULAR IMPRINTED POLYMERS FOR PHENYLBUTAZONE EXTRACTION

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Submitted: August 31, 2023 Revised: November 15, 2023 Accepted: January 31, 2024

ABSTRACT

Phenylbutazone is a pharmaceutical substance often added to rheumatic herbs. However, because of the complexity of the matrix caused by the presence of compounds in herbal medicines, the determination of phenylbutazone requires a time-consuming sample preparation process prior to analysis. This study was conducted to develop a specific sorbent that can be used to prepare phenylbutazone in herbal medicine. The performance of phenylbutazone Molecularly Imprinted Polymer (MIP) was evaluated using three distinct porogens (ethanol, methanol, and methanol-chloroform (1:1)) and two polymerization procedures (bulk and precipitation). According to the results of the polymer optimization, the polymer generated by precipitation in methanol-chloroform (1:1) has good sorbent characteristics. FTIR physical characterization revealed complete polymerization. The bulk procedure produces a more physically stable sorbent than the precipitation method does.

Keywords: MIP Performance Evaluation, Phenylbutazone Molecular Imprinted Polymer, Polymer Imprinting Factor

INTRODUCTION

Molecular Imprinted Polymer (MIP) are porous or hollow polymers with specific recognition sites for target molecules and the ability to recognize certain compounds. It has been widely used as a sorbent for solid-phase extraction (SPE). MIP is an adsorbent that can be used for pre-concentration, making sample analysis easier (Belbruno, 2019). MIP has good selectivity and can be utilized in sample preparation for phenylbutazone detection in herbal medicines. Phenylbutazone is a nonsteroidal anti-inflammatory medication (NSAID) that is often used to treat acute gouty arthritis and active rheumatoid arthritis (Engel et al., 2017). As a result, it is frequently abused or added to herbal products to enhance their medicinal efficacy. According to the BPOM public warning, herbal medicine or traditional medicine may not contain medicinal compounds. The addition of phenylbutazone to jamu is due to its benefits, which include low cost and clear anti-inflammatory therapeutic action. However, excessive phenylbutazone use can be detrimental to liver and renal function. Therefore, stricter monitoring of phenylbutazone in herbal medicines is required (BPOM, 2010). However, because of the complexity of the matrix in the presence of chemicals in herbal medicine, determining phenylbutazone frequently requires sophisticated sample preparation processes prior to analysis. The sample preparation approach using MIP can reduce the analysis time while increasing sensitivity and accuracy. To date, no MIP for phenylbutazone manufacturing has been developed in Indonesia.

As a result, the long-term goal of this research is to obtain an MIP material for solid-phase extraction column contents, abbreviated as SPE, which can selectively separate phenylbutazone from the interfering matrix, allowing for shorter, more accurate, and

selective monitoring of phenylbutazone abuse. The specific goals of this research included acquiring functional monomers as starting materials for sorbent manufacture, obtaining phenylbutazone sorbent materials, and obtaining the analytical and physical performance characteristics of the finished product.

RESEARCH METHODS

Equipment and Materials

Computational research

Hardware: HP netbooks with Intel AMD A6-7310 APU specifications with AMD Radeon R4 Graphics (4 CPUs), ~ 2.0GHz, 4GB RAM, Windows 10 Pro 64-bit system, **Software:** HyperChem 8.0.7 freeware with an internet connection.

Experimental research

Tools: Scanning Electron Microscopy- Energy Dispersion Spectroscopy (SEM-EDS) (JEOL JSM-6360 LA), Fourier-Transform Infrared Spectroscopy (FTIR) (prestige-21 Shimadzu), 250x4,0 mm Column (LiChrospher® 100 RP-18 (Merck), Solid Phase Extraction (SPE) Cartridge (Supelco column SPE superclean LC-Si), oven (Memmert), UV-Vis Spectrophotometer (Analytik Jenaspecord 200), digital analytical balance, and other laboratory glassware.

Materials: Phenylbutazone (TCI Chemical), Methampyrone (TCI Chemical), Acetaminophen (TCI Chemical), and glacial acetic acid (JT Baker and Fisher Scientific). Ethylene glycol dimethacrylate (EDGMA) (Aldrich), 2,2'-Azobis-2-methylpropionitrile (AIBN) (TCI Chemical), Methanol (Merck), Ethanol (Merck), Chloroform (Merck). Unless otherwise specified, all reagents used were of analytical grade.

Research Procedure

Monomer Computational Screening

Computational screening was performed using the ligand-ligand docking method on 20 types of monomers docked with the template (phenylbutazone) to determine the largest binding energy between the monomer and template, and on 3 types of crosslinkers docked with the template to determine the smallest binding energy between the crosslinker and template using Hyperchem freeware.

Constant Determination of Monomer-Template Association

UV-Vis spectrophotometry was used to determine the continuous association of template monomers. A UV spectrophotometer was used to quantify the concentration of phenylbutazone (2x10-5 M) in the methanol, ethanol, and methanol-chloroform (1:1) solutions. Then, 5x10-3 M methacrylic acid was gradually added. The absorption for each addition of methacrylic acid was measured, and the association constants were determined using the Benesi-Hildebrand equation:

$$\frac{1}{\Delta Y} = \frac{1}{Y\Delta HGKa[G]} + \frac{1}{Y\Delta HG}$$

Where ΔY is the change in absorbance, YΔHG is the change in absorbance with absorbance at the end of the titration, Ka is the association constant, and [G] is the concentration of the added monomer (Thordarson, 2011).

MIP Synthesis Using Bulk Polymerization

0.2663 grams of phenylbutazone (template) were dissolved in 10 mL of methanol-chloroform (1:1) in a closed vial and sonicated for 5 minutes until phenylbutazone were dissolved. A total of 340 L of methacrylic acid monomers (functional monomers) were added and sonicated for 20 minutes. 3.77 mL of Ethylene glycol dimethacrylate (EGDMA) as a crosslinker was added and sonicated for 5 minutes. As an initiator, up to 250 mg of 2,2'-Azobis-2-methylpropionitrile (AIBN) was introduced and sonicated for 20 minutes (until dissolved). The vial was sealed with parafilm and baked for 1 hour at 70°C. The vial was then placed in a water bath at 70 °C for 18 hours. The polymer was subsequently extracted for 24 hours with methanol:acetic acid (9:1) using a Soxhlet extractor. After extraction using

the Soxhlet method, the sorbent was dried in an oven at 50°C for 18 hours. A UV spectrophotometer was used to check that all phenylbutazone had been removed. Non-imprinted polymers (NIP) were created using the same technique as MIP, but without the inclusion of phenylbutazone (template).

MIP Synthesis Using Bulk Polymerization Precipitation Polymerization

Phenylbutazone (template, 0.2663 g) was dissolved in 50 mL of methanol-chloroform (1:1) in a closed glass bottle and sonicated until phenylbutazone was dissolved. After the addition of 340 L of methacrylic acid (functional monomers) and 300 mL of propanol/butanol, the mixture was sonicated for 20 minutes. 3.77 mL of Ethylene glycol dimethacrylate (EGDMA) (crosslinker) was added and sonicated for 5 minutes. As an initiator, 250 mg of azobis-2-methylpropionitrile (AIBN) was added and sonicated for 20 minutes (or until completely dissolved). The bottle was sealed with parafilm and baked for 1 hour at 70°C in an oven. The vial was placed in a shaker water bath at 70°C for 18 hours. The polymer was filtered and washed with 40 mL of methanol before drying in an oven at 50°C. The polymer was subsequently extracted using the same method as that used in the bulk method. Non-Imprinted Polymers (NIP) were created using the same technique as MIP, but without the inclusion of phenylbutazone (template).

MIP and NIP Adsorption Capacity Evaluation

Phenylbutazone solutions at concentrations of 4, 6, 8, 10, and 12 ppm were prepared. In the bulk technique, 5 mL of phenylbutazone solution of each concentration was added to a vial containing 20 mg MIP sorbent and 20 mg MIP sorbent precipitation method. After shaking the solution for 5 minutes, it was finished and left for 24 hours. After decanting the mixture, absorbance of the filtrate was measured using a UV spectrophotometer. The amount of phenylbutazone absorbed was determined by the difference between the initial and final concentrations of phenylbutazone in the filtrate. This evaluation was performed on the NIP in the same manner.

MIP and NIP Selectivity Determination

Selectivity was determined by adding phenylbutazone analog solutions containing methampyrone and acetaminophen. 5 mL of each solution (5 ppm) was placed in a separate vial containing 20 mg of MIP sorbent, shaken for 5 minutes, and allowed to stand for 24 hours. After decanting the mixture, absorbance of the filtrate was measured using a UV spectrophotometer.

Physical Characterization

Physical characterization of the polymer was carried out using FTIR and SEM.

RESULTS AND DISCUSSION

Monomer Computational Screening

As shown in Table I, methacrylic acid is a good and suitable functional monomer for polymerization in the fabrication of sorbents for the separation of phenylbutazone, whereas ethylene glycol dimethacrylate (EGDMA) is a good and compatible crosslinker. This can be seen in the ensuing free energy (E) value, where the lower the E value, the more spontaneous is the reaction or contact. Meanwhile, for crosslinkers, the opposite is true: the higher the E value, the less spontaneous is the interaction. Therefore, the crosslinker does not interfere with the performance of the functional monomers (Soni et al., 2018; Maulidya et al., 2023; Suryana et al., 2022).

Table I. Computational Screening Results

Functional Monomers		Bond Energy (kcal/mol)	Minimize energy (kcal/mol)	E value (ΔE)
Acid Monomer	Acrylic acid	-5562.6122225	-915.6485959	-1.3021071
	Methacrylate acid (MAA)	**-5847.8913241**	**-1197.8262884**	**-4.4035162**
	p-vinylbenzoic acid	-6774.0783656	-2124.7113073	-3.7055388

Functional Monomers		Bond Energy (kcal/mol)	Minimize energy (kcal/mol)	E value (ΔE)
Base Monomer	TFMAA	-5890.7765437	-1244.0234209	-1.0916033
	1-ethenylimidazole	-5987.7017643	-1342.0311869	-0.0090579
	2-(Diethylamino)ethyl methacrylate	-7665.9880083	-3018.7385450	-1.5879438
	2-vinylpyridine	-6273.2586886	-1627.5153011	-0.081868
	4-vinylpyridine	-6276.7200302	-1628.0289366	-3.0295741
	5-[1-(2,3-dimethylphenyl)etheny]-1H-imidazole	-7767.9280161	-3123.0484017	0.7819051
	Allylamine	-5622.2673950	-976.6552965	0.049421
	N-(2-Aminoethyl) methacrylamide	-6615.2483052	-1968.1483883	-1.4383974
	N,N_-diethyl-4-styrylamidine	-7985.1243576	-3339.5358682	0.0730301
	N,N-diethyl aminoethyl methacrylamide	-7733.3537285	-3087.0286333	-0.6635757
	N-vinylpyrrolidone	-6343.8798037	-1697.4245010	-0.7937832
	Urocanic ethyl ester	-6925.1473078	-2276.4491389	-3.0366494
Neutral Monomer	Acrylamide	-5616.9412386	-969.0666438	-2.2130753
	Acrylonitrile (AN)	-5378.3760167	-731.9001353	-0.8143619
	Ethylstyrene	-6957.3517779	-2310.3753189	-1.3149395
	Methacrylamide	-5903.3168497	-1254.2320903	-3.4232399
	Methyl Methacrylate (MMA)	-6111.8595879	-1464.5755261	-1.6225423
	Styrene	-6390.9452633	-1744.9706805	-0.3130633
	Trans-3-(3-pyridyl)-acrylic acid	-6656.1671607	-2010.0890100	-0.4166312

Cross-linker	Bond Energy (kcal/mol)	Minimize Energy (kcal/mol)	E value (ΔE)
EGDMA	**-9648.0640243**	**-5007.7455377**	**5.3430329**
N,N-methylenebisacrylamide	-6752.1817860	-2103.4268428	-3.0934237
Pentaerythritol triacrylate	-8618.1641621	-3973.6246147	1.1219721
Trimethylpropana trimethacrylate	-7457.6944732	-2811.4668930	-0.5660607

Constant Determination of Monomer-Template Association

According to the curves in Figure 1, Figure 2, and Figure 3, using a mixture of methanol and chloroform results in a higher Ka value. In methanol-chloroform solvent, the association constant between phenylbutazone and methacrylic acid is 2.9×10^3 M, which dictates the strength of the contact, and this Ka value determines the selectivity and specificity of the resultant polymer (Hasanah et al., 2020).

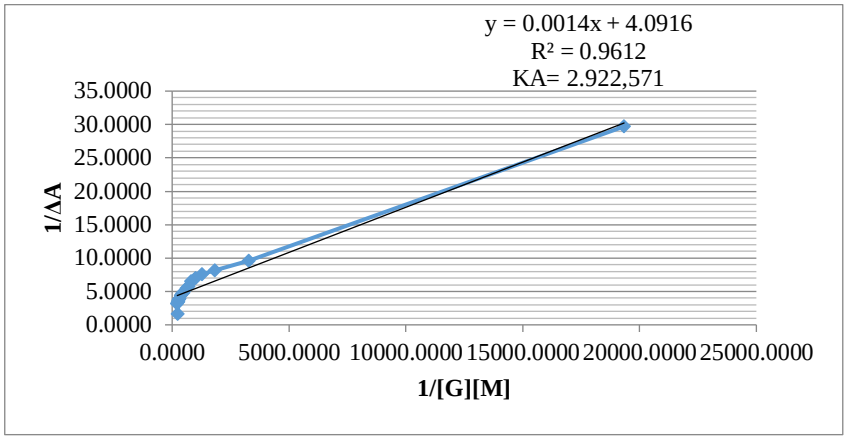

Figure 1. Association constant determination of phenylbutazone-methacrylic acid in methanol-chloroform (1:1)

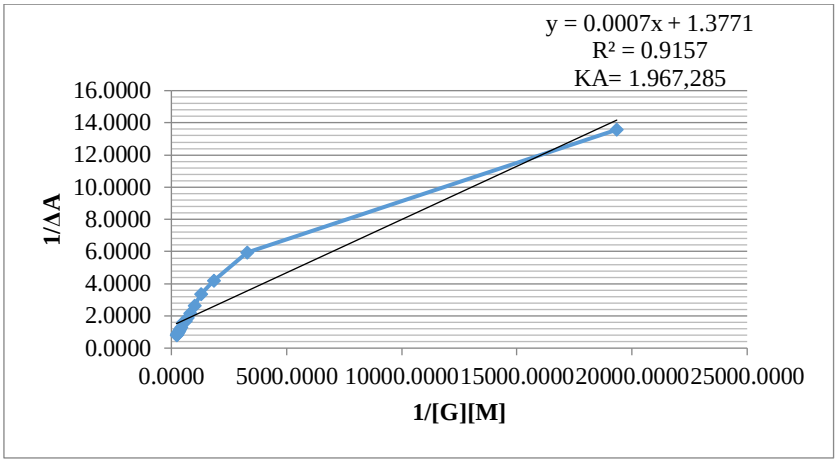

Figure 2. Association constant determination of phenylbutazone-methacrylic acid in ethanol

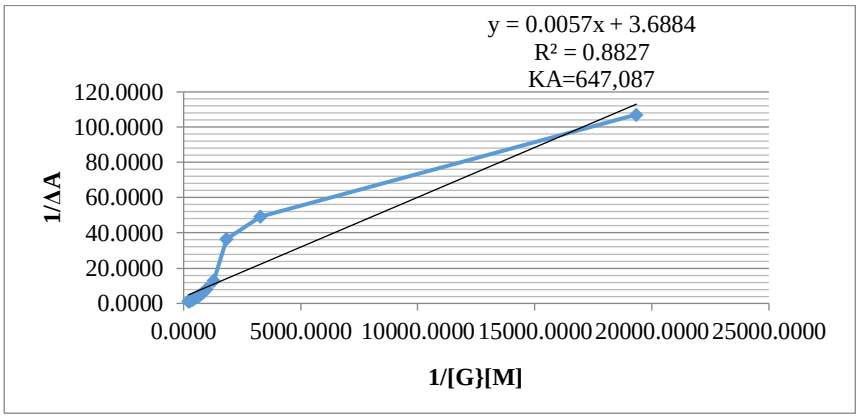

Figure 3. Association constant determination of phenylbutazone-methacrylic acid in methanol

Molecular Imprinted Polymer Synthesis

Bulk and precipitation polymerization methods were used. Phenylbutazone as a template was dissolved in methanol-chloroform (1:1) and methacrylic acid as a functional monomer, which was selected as a functional monomer based on computational testing, was added and sonicated for 5 minutes for the bulk approach. The mixture was sonicated for 40

minutes to remove oxygen before adding EGDMA as the crosslinker, which was chosen based on computational research, and AIBN as the reaction initiator. Polymerization was performed in an oven at 60 °C for 24 hours. The resulting polymer was crushed and sieved using a mesh size of 100. To test the retention of the resulting molecularly imprinted polymer (MIP), a molecular non-imprinted polymer (NIP) was created in the same manner but without the inclusion of phenylbutazone as a template. In terms of the deposition polymerization approach, NIP polymerization was carried out in a water bath shaker at 65°C for 24 hours (Suherman et al., 2018; Hasanah et al., 2019). The polymer was filtered and washed with methanol-chloroform (1:1) many times. The template molecule (phenylbutazone) was then extracted for 24 hours using Soxhlet extraction with chloroform containing 3% dissolved acetic acid to yield the MIP (Soni, 2020). The MIP was dried and preserved. The control polymer was prepared using the same method but without a die (NIP). The polymerization component composition employed was a generic formulation that is routinely used in the production of NIP polymers.

Table II. Ratio of Template:Monomer:Crosslinker in the Synthesis of MIP and NIP.

Ratio	Phenylbutazone (template)	Methacrylate acid	EGDMA (crosslinker)
MIP (1:4:20)	0,308 mmol	2,64 mmol	13,2 mmol
NIP (0:4:20)	-	2,64 mmol	13,2 mmol

MIP and NIP Adsorption Capacity Evaluation

The goal of determining good solvent conditions in the adsorption process was to evaluate the adsorption capabilities of the MIP and NIP adsorbents for phenylbutazone. These conditions were observed by analyzing the leftover phenylbutazone levels that were not bound to the sorbent using the batch approach utilized to analyze the binding sites on the MIP. In 10 mL volumetric flasks, up to 20 mg of methanol sorbent MIP was mixed with 5 mL of phenylbutazone solution at concentrations of 4, 6, 8, 10, and 12 ppm. After shaking for 10 minutes at 120 rpm, the mixture was allowed to stand for 18 hours at room temperature before filtration. A UV-Vis spectrophotometer was used to measure the absorbance of the filtrate. The amount of phenylbutazone absorbed was determined by subtracting the initial phenylbutazone concentration from the free phenylbutazone concentration in the filtrate. For comparison, the same technique was applied to the NIP sorbent. The Freundlich isotherm was used to plot the results.

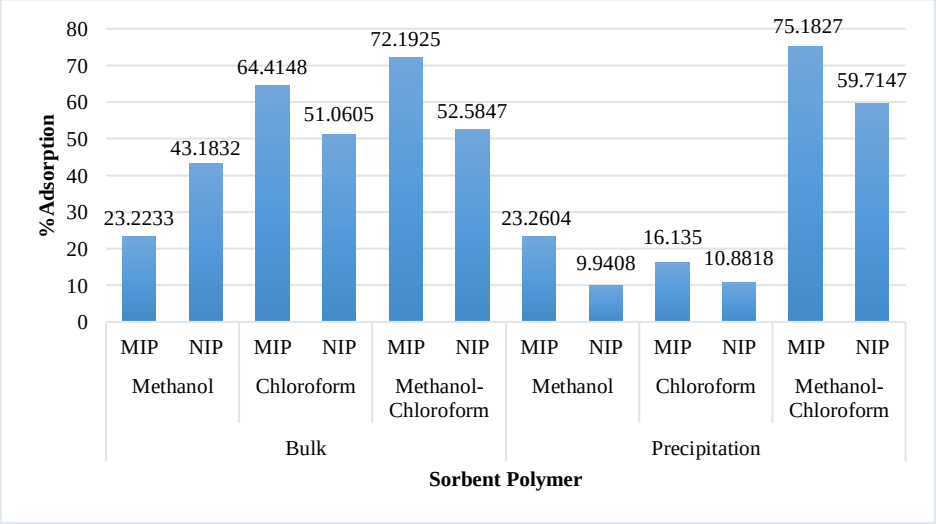

Figure 4. Comparison of % adsorption of PTM and NIP bulk polymerization and precipitation tested in different solvents

Figure 4 shows that the adsorption ability of the sorbent prepared by the precipitation polymerization technique for phenylbutazone has a higher percentage adsorption value than that of the sorbent prepared by the bulk polymerization technique, which is due to the sorbent made using the polymerization technique. Because the bulk creates a polymer in the form of chunks, it must be ground to minimize the size of the polymer generated. Consequently, grinding can harm the morphology of the sorbent. The most useful solvent is methanol:chloroform (1:1), which has the highest % adsorption value. Because the presence of chloroform reduces polarity, the hydrogen bonds produced are not overly disturbed (Hasanah et al., 2019).

The adsorption capacity was examined once it was determined that the solvent had high adsorption capacity. The purpose of this assessment was to determine the affinity and saturation capacity of the MIP binding to the template. The adsorption capacity was evaluated by computing the concentration of the bound analyte (B) and that of the free analyte (F) in a specific concentration range and plotting B against F to form a binding isotherm (Martín-Esteban, 2013). The Freundlich isotherm was chosen as the mathematical technique because it is ubiquitous and acceptable for use in heterogeneous MIPs because each template molecule has a variable absorption potential. This can aid in characterizing the properties of MIP and estimating the links between various bonding factors and affinity distributions. Non-covalent MIP synthesis results in heterogeneous cavities with varying affinities.

Table III. Freundlich Parameter of MIP and NIP in Methanol-Chloroform (1:1) as Porogenic Solvent

Polymer	Method	R	m	a (mg/g)
MIP 1	Bulk	0,9748	1,7857	0,5716
NIP 1	Bulk	0,9144	1,3455	0,2182
MIP 2	Precipitation	0.9769	2.8153	0.8518
NIP 2	Precipitation	0.9452	3.3818	0.5969

As shown in Table III, the sorbent produced by the precipitation polymerization approach had a higher capacity of 0.8518 mg phenylbutazone/gram sorbent than the sorbent produced by the bulk polymerization technique, which produced only 0.5716 mg phenylbutazone/gram sorbent. These data also show that MIP has a higher adsorption capacity than NIP, implying that the MIP sorbent has a higher adsorption capacity and binding affinity for phenylbutazone than the NIP sorbent. This is possible because MIP has a particular binding site for phenylbutazone, which NIP does not. The heterogeneity parameter (m) can be noticed in addition to parameter a. The presence of m at MIP and NIP 1 indicates that the system was neither homogeneous nor heterogeneous. The heterogeneous system means that the pore size and the phenylbutazone binding site possessed by the polymer are not the same size, resulting in the likelihood of uneven phenylbutazone binding. This uneven side is a consequence of the non-covalent synthesis process. This is because of the presence of template monomer complexes in the prepolymerization mixture as a result of several stoichiometric processes (Rachel, 2010).

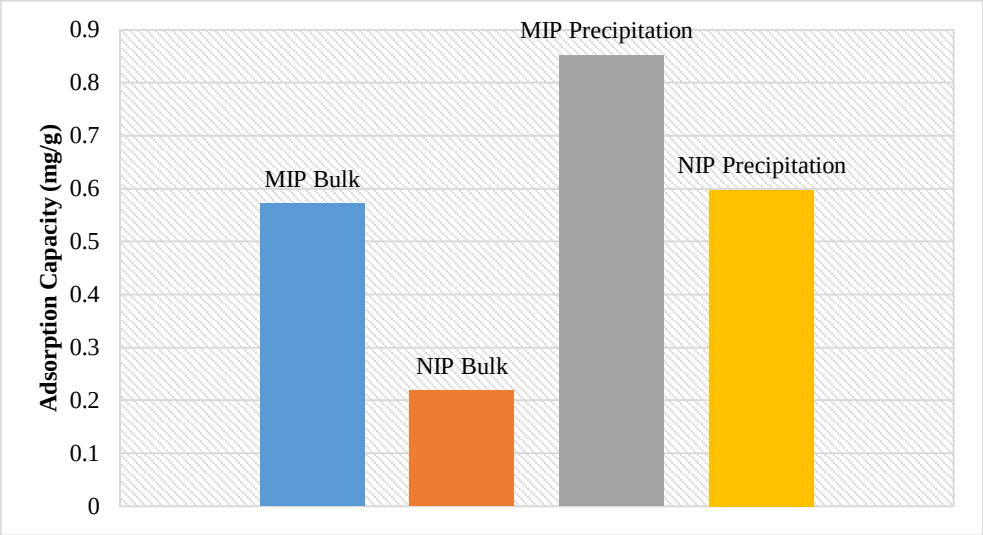

Figure 5. Comparison of adsorption capacity graph of bulk polymerization and precipitation methods

MIP and NIP Selectivity Determination

The next test was performed to determine the selectivity of MIP sorbents by comparing the distribution coefficient values of MIP sorbents to other medications with the same therapeutic goal. Acetaminophen and methampyrone were chosen as comparator medications Figure 4. MIP sorbent selectivity was determined by measuring the Imprinting Factor (IF), which is the ratio of the MIP and NIP distribution coefficients (KD). The IF value indicates the ability of the MIP prints to distinguish phenylbutazone from methampyrone and acetaminophen (Table IV).

According to Figure 5, MIP and NIP prepared by the bulk approach had absorption capacities of 0.5716 and 0.2182, respectively, whereas MIP and NIP prepared by the precipitation method had absorption capacities of 0.8518 and 0.5969, respectively. The sorbent produced by the precipitation polymerization process had the highest imprinting factor value against phenylbutazone. This demonstrates that the sorbent produced by precipitation polymerization has the highest selectivity compared with the sorbent produced by bulk polymerization. This is because unlike the bulk method, the deposition polymerization method does not experience erosion, so the identifier side is not damaged, and phenylbutazone produces a higher imprinting value than methampyrone and paracetamol because the print formed on the sorbent is identical to the phenylbutazone molecule (Hasanah et al., 2020).

Table IV. Sorbent Selectivity of MIP and NIP in Methanol-Chloroform (1:1) as Porogenic Solvent

Analyte	Method	Phenylbutazone	Methampyrone	Acetaminophen
KD MIP 1	Bulk	2,7778	4.0541	4.1705
KD NIP 1	Bulk	1,3522	3.0505	6.3816
Imprinting Factor	-	2.0302	1.3289	0.6535
KD MIP 2	Precipitation	3.125	4,5544	7,1376
KD NIP 2	Precipitation	1.2351	5,9259	7,1897
Imprinting Factor	-	2,5543	0,7685	0,6736

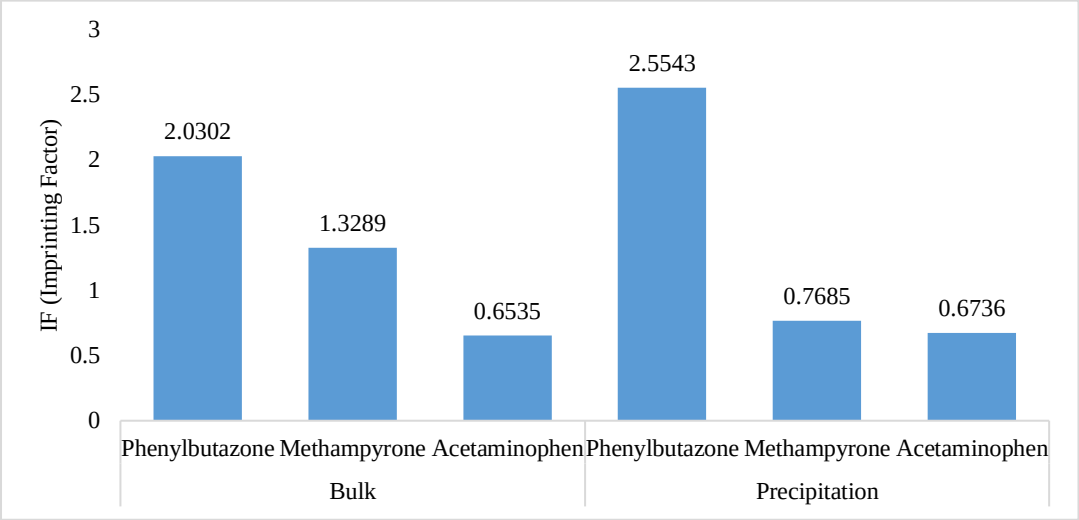

Figure 6. Graph of comparison of selectivity test results

Physical Characterization

Physical characterization tests were performed using FTIR and Scanning electron microscopy (SEM) to determine the physical form of the polymer before and after use as cartridges. FTIR spectroscopy was used to predict the functional groups and bond types in the chemicals, allowing for the physical characterization of MIP and NIP sorbents. Figure 6 shows that the FTIR spectra for the MIP and NIP polymerization reactions occur equally and perfectly because the spectra are comparable; notably, there is no absorption in the form of peaks in the area of approximately 1000 cm-1 from the vinyl group (Alotaibi et al., 2018).

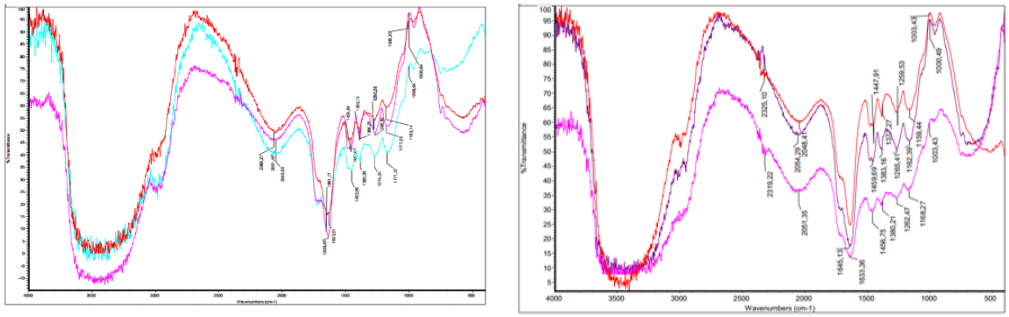

Figure 7. Overlay FTIR spectrum of polymer a) bulk polymer b) precipitation polymer; red = MIP sorbent before template extraction; green or purple = MIP sorbent after template extraction; pink = NIP sorbent

Scanning electron microscopy (SEM) was used to examine the polymer morphology (Figure 7). SEM was used to describe the geometry, particle size, and surface of the polymers (Arabzadeh and Abdouss, 2010). The results of the SEM characterization of MIP and NIP formed by bulk polymerization and precipitation revealed that the morphology of the MIP polymer displayed a hollow structure, in contrast to the NIP particles, which tended to be compact (Figure 8b and Figure 8d). Because the MIP prepared by precipitation polymerization contains cavities (Figure 8a)Figure 8 and smaller and more homogeneous particle sizes than the MIP made by bulk polymerization (Figure 8c), the MIP prepared by bulk polymerization has a larger surface area (Figure 8a).

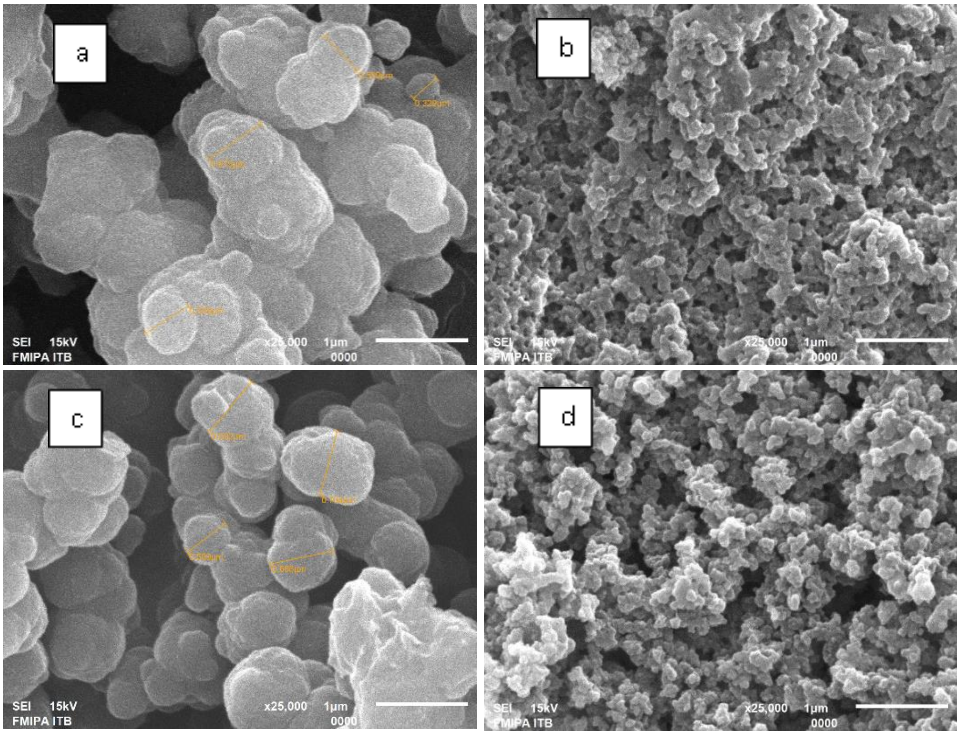

Figure 8. Polymer morphology was obtained by scanning electron microscopy at 25,000 times magnification. a. MIP bulk polymerization, b. MIP bulk polymerization, c. MIP precipitation polymerization, and d. MIP precipitation polymerization

CONCLUSION

According to the results of the computational screening, methacrylic acid is a good and acceptable functional monomer, whereas ethylene glycol dimethacrylate (EGDMA) is a good and compatible crosslinker. In addition, it can be used as a sorbent. Furthermore, experimental research using selected functional monomers and crosslinkers produced by two different polymerization methods revealed that the sorbent produced by the precipitation polymerization method outperformed that produced by the bulk approach in terms of phenylbutazone separation. The analytical performance of this sorbent can also be explored to separate phenylbutazone from herbal or traditional medicine samples using solid-phase extraction procedures in the future.

ACKNOWLEDGMENT

The authors thank The Ministry of Research and Technology and Higher Education of the Republic of Indonesia, LLDIKTI IV, Universitas Garut for research funding trough Penelitian Dosen Pemula (PDP) tahun 2023.

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