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Original Article

SYNTHESIS OF MOLECULAR IMPRINTED POLYMER SALBUTAMOL USING METHACRYLIC ACID MONOMER AND TRIMETHYL PROPANE TRIMETHACRYLATE (TRIM) AS A CROSS-LINKER THROUGH SUSPENSION POLYMERIZATION

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ABSTRACT

Objective: This study aims to determine the analytical performances and characteristics of MIP salbutamol made with methacrylic acid (MAA) monomer and trimethylpropane trimethacrylate (TRIM) cross-linker through suspension polymerization.

Methods: The MIP salbutamol was synthesized using suspension polymerization. The analytical performances of MIP, such as the adsorption ability, adsorption capacity and selectivity, were evaluated by Spectrophotometer UV-Vis. The physical characterization of MIP and NIP were evaluated using FTIR, TEM-EDS, Brunauer-Emmett-Teller (BET) method and Barret-Joyner-Halenda (BJH) method.

Results: Molecular Imprinted Polymer (MIP) showed better analytical performance than Non-Imprinted Polymer (NIP), the adsorption ability of MIP and NIP reached about 90.43% and 53.92%, respectively. The MIP was selective for salbutamol when compared to terbutaline and salmeterol xinafoate with an imprinting factor (IF) of 1.2841. The MIP has spherical shape particles with diameters in the range of 10-100 µm with a surface area of 185.546 m^2/g, pore volume of 0.257 cm^3/g, and pore size of 16.599 Å.

Conclusion: The Based on these results, MIP salbutamol, has the potential to be developed as a method for the preparation of salbutamol analysis from biological samples.

Keywords: Salbutamol, Molecularly imprinted**,** Suspension polymerization, Separation

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INTRODUCTION

Salbutamol, a beta-2 agonist drug, is a drug used to relieve asthma symptoms by relaxing the muscles that cause the narrowing of the airways to the lungs. This drug is also often used to relieve cough in acute [1]. However, salbutamol is often abused by several parties. Salbutamol has an anabolic effect so the use of oral salbutamol by athletes is feared to be misused as a doping drug or to increase endurance and strength. In 2010, the World Anti-Doping Agency (WADA) added that the use of beta-2 agonist drugs is prohibited except for inhalation of salbutamol with a maximum use of 1600 µg for 24 h. WADA stipulates the maximum level of salbutamol in urine is 1000 ng/ml [2]. Moreover, beta-2 agonist drugs, including salbutamol are also often used illegally to stimulate the growth of meat-producing animals. Residues of beta-2 agonists in the liver and meat of animals are harmful to humans [3]. Therefore, the Indonesian government has banned the use of beta-2 agonist drugs in livestock. This prohibition is stated in the Regulation of the Minister of Agriculture number 14 of 2017 concerning the veterinary drug's classification [4].

From the explanation above, it can be concluded that there is a need for a tool or method that can be used to identify and quantify salbutamol in particular to detect the illegal use of this drug. Several methods have been developed to analyze salbutamol, such as high-performance liquid chromatography (HPLC) [5], liquid chromatography-mass spectrometry (LC-MS) [6], capillary electrophoreses [7], gas chromatography-mass spectrometry (GC-MS) [8], and enzyme immunoassay [9]. However, the instrumentation method shows various weaknesses, one of which is the complicated sample pre-treatment process, especially in separating the target molecule from a complex sample or matrix [10, 11].

Therefore, we need a method that can be a solution to overcome the problems in the pre-treatment process. One method that can be developed is Molecular Imprinted Polymer (MIP). MIP is a polymer synthesized based on the formation of a complementary recognition cavity induced by the template, which is thus made specific in shape, size, and functionality to the target chemical or biological molecule [12]. MIP can be a promising alternative because of its simple synthesis step, high sensitivity and specificity, comparable performance to natural bio-receptors, high stability, and low cost [11].

Several studies used to synthesize salbutamol MIP were bulk polymerization [13] and precipitation polymerization [3]. However, no studies are currently showing the synthesis of salbutamol MIP using suspension polymerization. Suspension polymerization is a polymerization synthesis carried out in a heterophase medium, where the reactive phase (monomer) is insoluble in the continuous phase (usually water). This polymerization is easy and simple to operate; the minimum amount of contaminants in the final product. The particle size ranges obtained from suspension polymerization ranging from 50-500 µm, which can be easily controlled by a combination of stirring speed and suspending agent concentration [14]. Suspension polymerization is also one of the methods that can be applied on a large scale [15].

The factors that influence the result of MIP synthesis besides the polymerization method were the MIP component consisting of templates, functional monomers, cross-linkers, porogens, and initiators [16]. Methacrylic acid was used as a monomer functional because it has a carboxyl group that can act as a hydrogen donor and acceptor simultaneously [17, 18]. This allows a strong interaction between the template and the monomer through hydrogen bonds formed from the hydrogen atom in the–COOH group of methacrylic acid with the oxygen atom in the C=O group of salbutamol [19].

Cross-linker used in this study was TRIM because it provides a polymer with more rigidity and more effective binding sites compared to ethylene glycol dimethacrylate (EGDMA) [20]. The study conducted by Pangkamta *et al.*, (2020) also showed that MIP using TRIM had higher binding than MIP using EGDMA [21].

Therefore, this study aimed to synthesis the MIP salbutamol with methacrylic acid (MAA) monomer and trimethylpropane trimethacrylate (TRIM) cross-linker through suspension polymerization, and determined its analytical performances.

MATERIALS AND METHODS

Material

Salbutamol sulphate was obtained from Supriya Lifescience LTD, India. Terbutaline sulfate was obtained from LKT Lab. Salmeterol sulfate and trimethylpropane trimethacrylate (TRIM) were purchased from TCI. Methacrylic acid, azobisisobutyronitrile (AIBN), and polyvinyl alcohol (PVA) were purchased from Sigma Aldrich. HPLC-grade methanol and potassium bromide were obtained from Merck. HPLC grade acetonitrile was purchased from J. T Beker. If not otherwise specified, all chemicals are analytical grade.

Methods

Synthesis of MIP and NIP using suspension polymerization method

The polymerization method was carried out with several modifications based on the method in Zhang and Lei's (2013) research [22]. MIP synthesis was carried out using salbutamol as a template, methacrylic acid as a monomer, and TRIM as a cross-linker with moles ratio of 1:4:20. The composition of the materials is shown in table 1. Salbutamol and methacrylic acid were dissolved in 2.5 ml of methanol, then sonicated for 5 min. Then, TRIM and AIBN were added and sonicated for 5 min. The mixed solution was added to 1.5% w/v PVA dissolved in 25 ml of water. The mixed solution was sonicated again for 10 min. After sonication, the mixed solution was placed in an oven at 70 °C for 1 h. After that, it was stirred using a magnetic stirrer while heating at a temperature of 70 °C for 24 h. The obtained polymer was filtered and rinsed with methanol to remove any remaining reagent. The polymer was then dried in an oven at 50 °C for 24 h. The dried polymer was ground and sieved using 80 mesh. NIP was made using the same procedure but without adding a template. Template extraction from MIP was carried out using the soxhlet extraction method with a mixture of methanol: acetic acid with a ratio of 9:1 v/v.

Adsorption ability evaluation

The standard solutions of salbutamol 5 mg/l were prepared in several solvents: water, methanol, water: methanol (9:1), and water: acetonitrile (9:1). Then, each 5 ml of the solution was added to a vial containing 20 mg of MIP. The mixture was then agitated for 5 min and allowed to stand for 24 h. The filtrate was measured using a UV-Vis spectrophotometer. The adsorption ability was calculated from the difference between the initial and final concentrations of salbutamol in the filtrate. This work was also carried out on NIP with the same procedure [23].

Table 1: Composition of materials that are used in the synthesis of MIP and NIP

Polymer	Ratio	Salbutamol (mg)	Methacrylic Acid (ml)	TRIM (ml)
MIP	1:4:20	23.9	0.034	0.638
NIP	0:4:20	-	0.034	0.638

Adsorption capacity evaluation

The standard solutions of salbutamol with a concentration of 2.5; 5; 7.5; 10; and 12.5 ppm were prepared. Then each 5 ml of the solution was put into a vial containing 20 mg of MIP. The mixture was then agitated for 5 min and allowed to stand for 24 h. The filtrate was measured using a UV-Vis spectrophotometer. The data obtained were plotted into the Freundlich and Langmuir isotherm adsorption curves. This work was also carried out on NIP in the same procedure [23, 24].

MIP selectivity evaluation

MIP selectivity was determined by preparing salbutamol, terbutaline and salmeterol xinafoate standard solutions with a concentration of 5 mg/l for each solution. 5 ml of each solution was put into the different vial containing 20 mg of MIP and agitated for 5 min. The mixture allowed to stand for 24 h. The mixture was decanted and the absorbance of the filtrate was measured using a UV-Vis spectrophotometer. The distribution coefficient (K_D) and imprinting factor (IF) were calculated. This work was also carried out on NIP in the same stages [23, 24].

Physical characterization

The MIP (2 mg) was crushed with 198 mg of potassium bromide and then molded into a pellet. The infrared spectrum of MIP was observed using the Fourier Transform Infrared (FTIR) instrument at a wave number of 4000-400 cm^{-1}. The determination of the MIP functional group was carried out before and after extraction. The same procedure is also carried out on the NIP. In addition, characterization was also carried out using Transmission Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (TEM-EDS) to observe polymer morphology [25, 26]. Brunauer–Emmett–Teller (BET) theory was used to determine surface area, and Barrett–Joyner–Halenda (BJH) theory was used to calculate pore volume total.

RESULTS AND DISCUSSION

Synthesis of MIP and NIP using suspension polymerization method

In suspension polymerization, a stabilizer was used to maintain the stability of the monomer/polymer particle dispersion from incorporation in the continuous phase (water) [27]. In this study, the stabilizer used is polyvinyl alcohol. Methacrylic acid used as a monomer, has a carboxyl group and can act as a hydrogen donor and acceptor at the same time [17, 18]. It facilitated a strong interaction between the template and monomer through non-covalent bonds. The hydrogen bonds formed from the hydrogen atom in the-COOH group from methacrylic acid with the oxygen atom in the C=O group and the nitrogen atom in the secondary amine group (N-H) of salbutamol [19]. The interaction scheme between salbutamol and methacrylic acid can be seen in fig. 1. TRIM has three branches containing three vinyl groups in the molecule, which may be a favorable structure for polymerization that would result in a more rigid complement recognition site on the template [21, 28].

Adsorption ability evaluation

Evaluation of adsorption ability was carried out to determine the optimal solvent conditions so that the polymer could well adsorb the analyte [29] because the swelling ability of MIP was assumed to be different in different solvents and could affect the binding site affinity of MIP [30]. Fig. 2 showed the adsorption ability of MIP and NIP in various solvents. The percentage of MIP adsorption ability for all solvents was greater than NIP, indicating that the salbutamol's printing process on MIP has succeeded. The highest adsorption ability of MIP and NIP were found in water solvent with %adsorption of 90.43±3.03% for MIP and 53.92±8.01% for NIP. The imprinting factor (IF) value in water solvents was also the highest among IF in other solvents (IF=8.07). The IF value can describe the quality of the binding site formed on the MIP [31]. Water has a high hydrogen bonding capacity which can prevent the formation of salbutamol-salbutamol complexes in solution [32, 33]. This may promote the formation of a bond between active site of MIP and salbutamol. The higher adsorption value in water is most likely due to the swelling effect of the polymer which is more suitable under aqueous conditions [33].

Adsorption capacity evaluation

The adsorption capacity was evaluated to see the affinity between MIP and salbutamol. The adsorption capacity can be determined using an isotherm adsorption model. The adsorption isotherm model is a source of essential information about the adsorption process [34]. The adsorption isotherm model used in this study is the Freundlich and Langmuir isotherm model. This model can explain the interaction mechanism between the analyte and the sorbent surface [30]. The result of this study has shown in table 2.

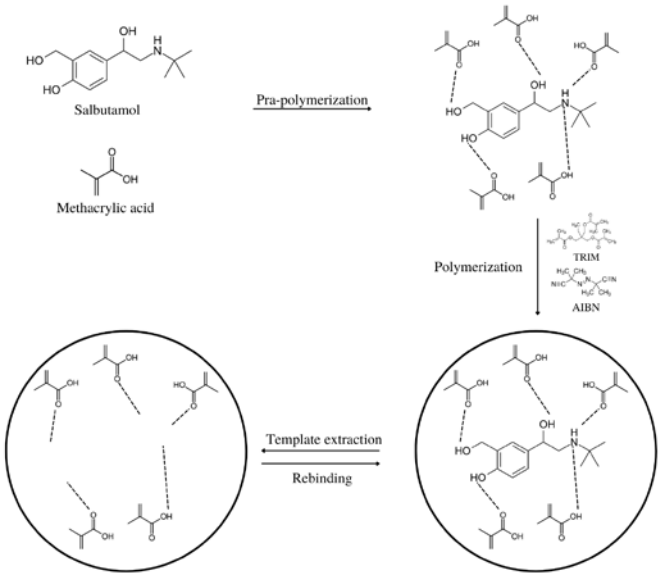


Fig. 1: The synthesis scheme of MIP

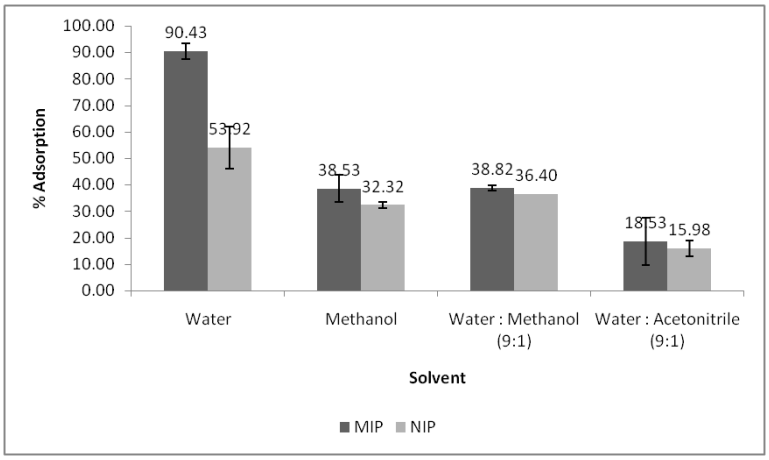


Fig. 2: Result of adsorption ability evaluation for MIP and NIP

Table 2: Result of adsorption capacity evaluation for MIP and NIP using Isotherm freundlich and langmuir model (n = 3)

Polymer	Isotherm freundlich			Isotherm langmuir		
	a (mg/g)	m	R^2	qm (mg/g)	Ke	R^2
MIP	0.3316	0.6679	0.9317	2.0593	0.1822	0.8699
NIP	0.6157	0.2568	0.9353	1.2228	0.6534	0.9873

Note: a = Adsorption capacity (mg/g), m = homogeneity index, qm = adsorption capacity (mg/g), Ke = Langmuir constant, R^2 = correlation coefficient.

Table 2 showed that MIP has a higher correlation coefficient (R^2) in the Freundlich isotherm model, with an R^2 value of 0.9317, indicating that the analyte adsorption process by MIP takes place following the Freundlich isotherm model, which is a heterogeneous and multilayer. Meanwhile, the Langmuir isotherm model was the best fit model for NIP with an R^2 value of 0.9873. In NIP, the adsorbate adsorption process was homogeneous and occurs in a single layer (monolayer) of adsorbent molecules [35].

In the Freundlich isotherm model, there is a homogeneity parameter (m). A homogeneity index value close to 1 indicates that the adsorption occurs homogeneous, while a value close to 0 indicates that the adsorption is not homogeneous [36]. The higher the homogeneity index value, the more homogeneous and better the adsorption ability [30]. MIP homogeneity value was 0.6679 and for NIP was 0.2568. The homogeneity values of MIP and NIP was not close to 1, indicating that MIP and NIP have an inhomogeneous adsorption system. However, if viewed from the value, the homogeneity index of MIP was greater than NIP, which showed that the analyte adsorption process by MIP was more homogeneous than NIP.

The adsorption quantity of MIP can be calculated according to the equation:

$$Q = \frac{(C0 - C)V}{W}$$

Where Co (mg/l) and c (mg/l) are the initial and equilibrium concentration of salbutamol, respectively. V (ml) is the volume of the standard solution and W (mg) is the weight of MIP or NIP [24].

The adsorption quantity of MIPs was 1.161 mg/g at a salbutamol concentration of 12.5 mg/l. comparing the study conducted by

Jun-Bo, *et al.* (2014), the adsorption quantity of MIPs was 7.33 mg/g at a salbutamol concentration of 100 mg/l. We cannot compare the results because the concentrations used are different. The concentration of the analyte would influence the adsorption quantity when the analyte was not sufficient to saturate the specific binding cavities of the MIP [10]. Therefore, the salbutamol concentration of 12.5 mg/l used in this study may not be sufficient to saturate the MIP, it is causing a low adsorption quantity of MIP, so analysis using a higher concentration is required to determine it.

MIP selectivity evaluation

The determination of MIP selectivity can be concluded by comparing the binding of the analyte with structural analogues under the same adsorption conditions [30]. The parameters used in determining the selectivity are the distribution coefficient (K_D) and the imprinting factor (IF). KD is the ratio of the amount of analyte adsorbed to the concentration of analyte in the solution, while IF describes the quality of the impression site formed on MIP sorbents [31]. The result of MIP selectivity evaluation can be seen in table 3.

Table 3: Result of selectivity MIP dan NIP (n = 3)

Compound	Distribution coefficient (K_D)±SD		Imprinting factor (IF)±SD
	MIP	NIP	
Salbutamol	352.07±24.35	274.99±33.48	1.29±0.11
Terbutaline	496.38±92.03	558.55±140.08	0.92±0.24
Salmeterol Xinafoate	595.68±108.92	1086.28±126.98	0.55±0.04

The K_D MIP value for salbutamol was smaller than for terbutaline and salmeterol. This shows that MIP adsorbs salbutamol less than terbutaline and salmeterol. The same thing happened to NIP. The K_D value can represent the amount of analyte adsorbed by MIP and NIP. Therefore, larger amounts of MIP are needed to be able to adsorb more salbutamol. However, the IF value of salbutamol was higher than terbutaline and salmeterol. The IF value can describe the quality of the print site on the MIP. A good IF value is more than 1, which indicates a better MIP imprint site than NIP [16].

Physical characterization

Characterization using FTIR

Using FTIR, characterization was performed to identify the functional groups present in MIP and the functional groups responsible for the interaction between MIP and the template. The MIP spectrum was compared with the NIP spectrum as a control of MIP [37]. Characterization using FTIR was carried out on MIP before and after extraction, as well as NIP, to see the difference in the spectrum results obtained. The results of the FTIR spectrum can be seen in fig. 3. The spectrums showed that MIP and NIP have identical functional groups, such as a sharp peak at wave number 1700 cm^{-1} indicating the carbonyl group (C=O) of methacrylic acid and TRIM. In addition, there is a-OH peak at a wave number of 3400-3650 cm^{-1} as a marker of the carboxylic group (-COOH) contained in the methacrylic acid monomer [18]. The presence of C-H and-CH_2 peaks at 2900 cm^{-1} and 1400 cm^{-1} as a marker of the methylene group of methacrylic acid [31]. The peak of the C=O group and the-OH group in the IR spectrum was also shown in the IR spectrum of MIP salbutamol synthesized by Jun-Bo, *et al.* (2014) [24].

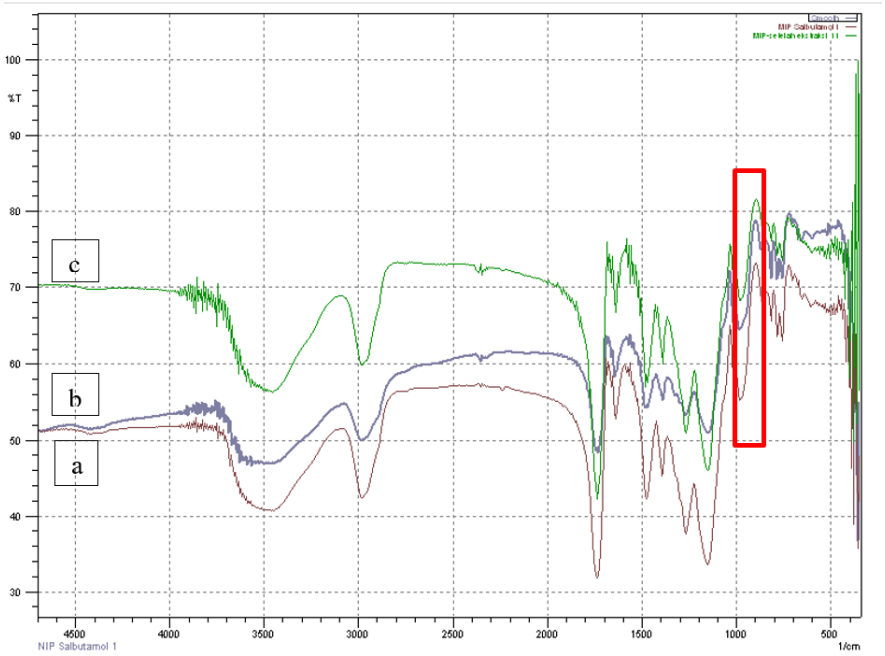
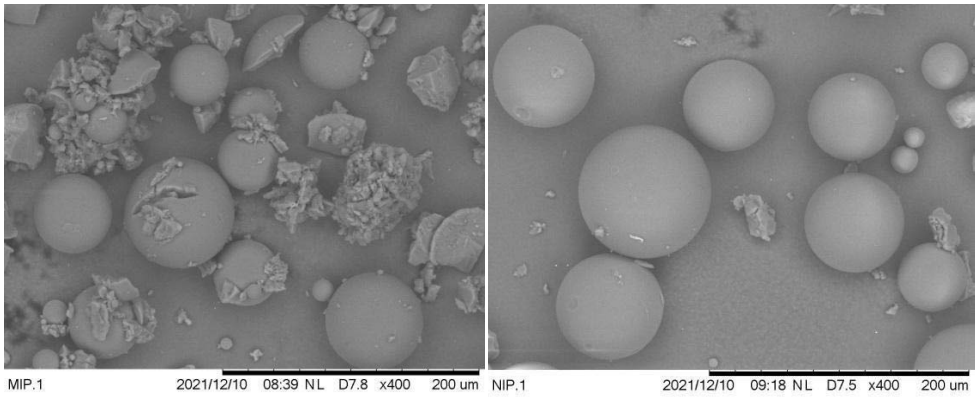


Fig. 3: Infrared spectrum (a) MIP before extraction; (b) NIP; (c) MIP after extraction

From the spectrum obtained, it can be seen that the polymerization has occurred ideally, which is indicated by the absence of twin vinyl peaks of methacrylic acid at a wavelength of 900-1000 cm^1, characterized by a red box [37].

Characterization using TEM-EDS

The morphology of MIP and NIP was observed using Transmission Electron Microscopy (TEM) at 400x magnification. The results of the physical characterization of MIP and NIP are shown in fig. 4.

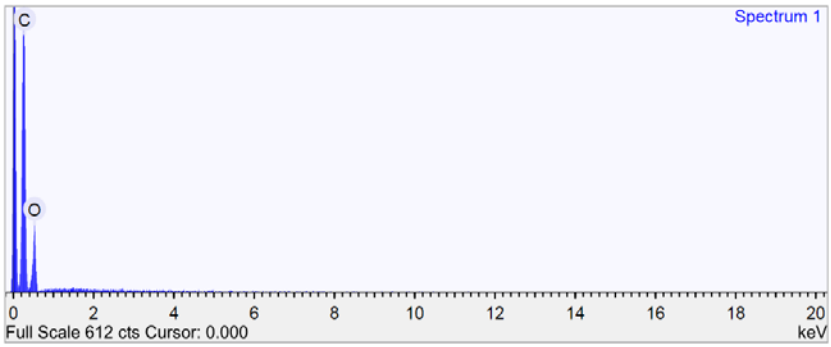


(a) **(b)**

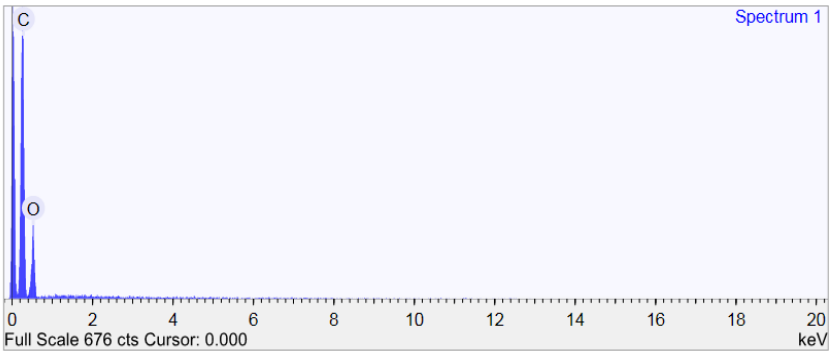
Fig. 4: TEM micrograph with 400x magnification of a) MIP and b) NIP

The picture above shows that the synthesized MIP and NIP have a spherical shape and the particle size of MIP and NIP varies. MIP and NIP diameters are in the range of 10-100 µm. MIP with a spherical shape and size is suitable for a sorbent in solid-phase extraction [38]. The results above also show that the presence of templates in the polymerization process does not significantly affect the particle morphology and physical characteristics of MIP [38].

Energy Dispersive X-Ray Spectroscopy (EDS) was used to analyze the atomic elements in the MIP and NIP formed [26]. The EDS spectrum of MIP and NIP can be seen in fig. 5.



(a)



(b)

Fig. 5: Spectrum of EDS a) MIP b) NIP

From fig. 5, synthesized MIP and NIP had carbon (C) and oxygen (O) atoms, with the percentage by weight (%wt) listed in table 3. These EDS data are appropriate and support the characterization using FTIR, which shows that there are functional groups formed by C and/or O atoms.

Table 3: Characterization of MIP and NIP using EDS (n=1)

Polymer	Atom	
	C (%wt)	O (%wt)
MIP	62.445	37.555
NIP	60.518	39.482

Nitrogen adsorption-desorption analysis

The surface area of the sorbent was measured using the Brunauer-Emmett-Teller (BET) method, and the pore volume and pore size were measured using the Barret-Joyner-Halenda (BJH) method. Table 4 show that the BET surface area and BJH pore volume of NIP are higher than MIP. In most cases, MIP has higher BET surface area and BJH pore volume that result in stronger of adsorption ability. However, in this study, even though BET surface area and BJH pore volume of MIP are lower than NIP, the adsorption ability MIP was better than NIP. It is because the MIP has molecularly imprinted sites that can improve the binding specificity of MIP in line with its higher pore size [39].

Table 4: Surface area and total pore volume of MIP and NIP

Sorbent	BET surface area (m^2/g)	Pore volume BJH (cm^3/g)	Pore size (Å)
MIP	185.546	0.257	16.599
NIP	373.954	0.287	15.592

CONCLUSION

The sorbent of MIP of salbutamol has been synthesized by suspension method using methacrylic acid as a monomer and trimethyl propane trimethacrylate as a cross-linker. The result show that MIP salbutamol has a better adsorption ability than its NIP. The MIP has good selective for salbutamol when compared to terbutaline and salmeterol xinafoate. Therefore, this MIP is a promising sorbent that can be used for the process of separating salbutamol in samples. However, further study is needed to determine the ability of MIP to separate salbutamol in various matrix samples.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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