Molecular Imprinted Polymer for Ethylmorphine with Methacrylic Acid and Acrylamide as Functional Monomer in Butanol Using Two Polymerization Method

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Abstract: Ethylmorphine is an opioid that has therapeutic effects as narcotic analgesic and antitussive, which has low levels and can be misused. Hence, it is crucial to monitor by analyze the levels of ethylmorphine in blood selectively. The preparation method that can be used to extract ethylmorphine from the sample is using molecular imprinting solid-phase extraction (MI-SPE) due to its sensitivity and selectivity. This study aims to compare the result of synthesis using two different polymerization methods, and also to examine the analytical performance and characteristics of imprinted polymers from two distinct functional monomers: methacrylic acid (MAA) and acrylamide (AM). The stages of this study include the determination of association constants, synthesis of polymer MI-SPE ethylmorphine using bulk and precipitation polymerization method, extracted template from the polymer, and determined the adsorption ability, capacity, and selectivity of the polymer. MI-SPE that has been made then characterized by using Fourier-Transform Infrared (FTIR) and Scanning Electron Microscope (SEM). The results showed that MIP with acrylamide (MIP-AM) as functional monomer and made by precipitation polymerization had better analytic performances than MIP that made by bulk polymerization, with affinity value 0.072 mg/g and homogeneity value -0.77. It is also selective toward ethylmorphine with imprinting factor value 27.43. In addition, the result of characterization using FTIR and SEM showed that MIP-AM 2, MIP-MAA 1, and MIP-MAA 2 might have a low degree of polymerization due to the presence of vinyl peaks, besides MIP-AM 2 and MIP-MAA 2 had smaller particle size than the NIP with an average value of $0,31 \pm 0,21$ μm and 0.28 ± 0.05 μm. Based on the result of this study, MIP-AM made by precipitation polymerization could be used to extract ethylmorphine on solid-phase extraction.

Keywords: ethylmorphine; molecularly imprinted polymer; solid-phase extraction; acrylamide; methacrylic acid.

1. Introduction

Ethylmorphine is one of the morphine derivatives which has pharmacological effects as analgesic and antitussive. In Europe, ethylmorphine was approved as a drug for dry cough [1]. However, treatment with ethylmorphine can allow for side effects, abuse, and addiction [2]. According to Regulation of the Minister of Health of Republic of Indonesia Number 20 of 2018, ethylmorphine belongs to group III narcotics which has medicinal properties and widely used in therapy and/or science development purposes and has mild potential to cause addiction [3]. A Swedish study of medicolegal autopsies reported 14 cases of misuse of antitussive drugs containing ethylmorphine by alcoholics and drug addicts. In the analysis, it was obtained that the use of overdose ethylmorphine with blood concentrations above the therapeutic range ($\geq$ 0.3 μg/g) can cause fatal poisoning and death [4]. Another study case mentioned the correlation of ehtylmorphine consumption with the death of a baby who consumed ethylmorphine in antitussive drugs [5].

The qualitative and quantitative analysis of ethylmorphine from biological samples is quite complicated because its concentration is minimal in complex sample matrices [6,7]. Therefore, selective sample preparation methods and sensitive analytical methods are needed to detect and quantify samples [8]. The analytical method which is widely used to determine ethylmorphine level is Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) because it is relatively fast, sensitive, and selective. However, biological samples generally cannot be directly analyzed by UPLC-MS/MS; sample preparation is needed to obtain accurate result [9,10]. The preparation method that is currently developing is molecularly imprinted solid-phase extraction (MI-SPE) because of its high selectivity and affinity to the desired molecule [11].

Molecular imprinting technology (MIP) is a technique to create recognition sites for specific analytes in synthetic polymers. The recognition sites artificially have shape, size, and chemical function that complements the analyte [12]. The MIP components consist of monomer, cross-linker, initiator, and porogen. Monomer must be able to interact with template to form specific donor-receptor complexes in polymerization. Commonly used monomers in MIP are acrylamide and methacrylic acid. Acrylamide can form strong hydrogen bonds with a model. Stronger interactions between templates and monomers produce MIP with better capacity and selectivity [13].

Meanwhile, methacrylic acid can also give a dimerization reaction that can increase the imprinting effect [14]. The porogen that is usually used in MIP with hydrogen bond interactions is a solvent with a low dielectric constant, typically a solvent that tends to be non-polar. The use of non-polar solvents was chosen because if polar solvents were used, they can interfere the hydrogen bond of monomer-template. One of the fairly low polarity solvent is butanol [15], butanol were choose as ethylmorphine only dissolved in solvent with fairly to high polarity index.

There are various polymerization methods including bulk, precipitation, suspension, swelling, surface, and in situ polymerization. Each method has advantages and disadvantages. Bulk polymerization is the most often used method to synthesize MIP because the process is relatively simple, and the reaction is easy to control while precipitation polymerization can produce uniform microsphere MIP particles in one step polymerization [16].

Currently, no literature shows a study on molecular imprinted polymer for sample preparation to measure ethylmorphine levels in the biological sample. Therefore, this study aimed to synthesize sorbent based on molecular imprinting for ethylmorphine with acrylamide and methacrylic acid as monomer and butanol as porogen using bulk and precipitation polymerization method to compare the effectiveness of both methods. Further, the analytical performance and physical characterization of the sorbent was evaluated.

2. Results and Discussion

2.1. Determination of Monomer-Template Association Constants Using UV-Vis Spectrophotometers

The strength of interaction and complex formation between monomers and template are an important thing to be determined because it will represent the affinity and selectivity of the synthesized polymers [17]. Therefore, it was necessary to know how strong the interactions to form a stable complex in the pre-polymerization solution through the determination of association constants (Ka) [18,19].
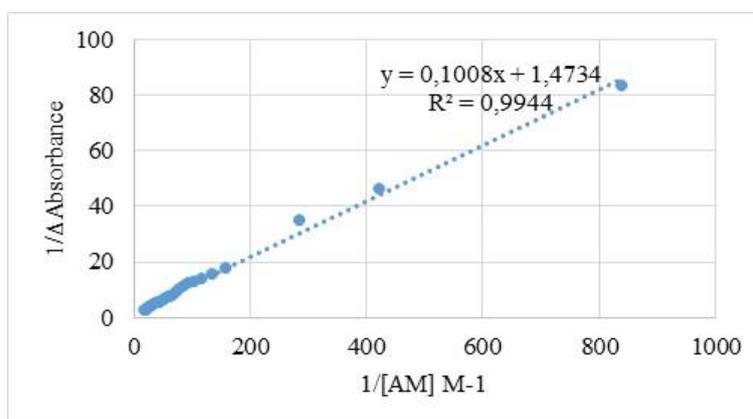
Figure 1. Relation curve between 1/[AM] to 1/ΔAbsorbance
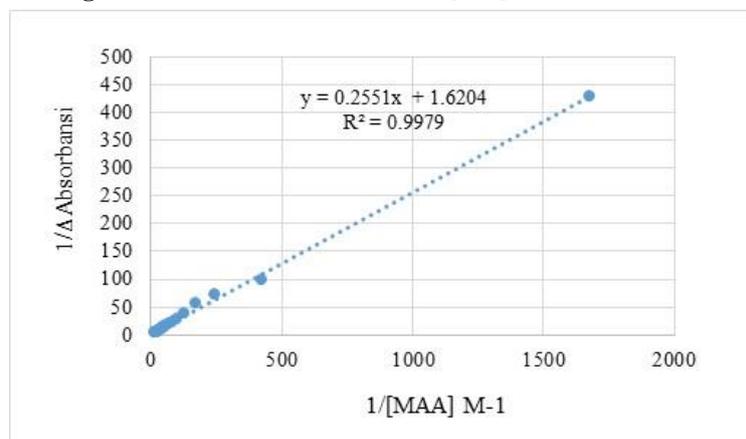
Figure 2. Relation curve between 1/[MAA] to 1/ΔAbsorbance

The association constants were determined by titration method using UV-Vis spectrophotometer. The change in absorbance of the solution was measured at each addition of the monomer solutions. The measurement results were plotted on a curve between 1/[Monomers], which were added as the x-axis and 1/ΔAbsorbance as the y-axis [20], as shown in Figure 1 and Figure 2. The value of the association constants was calculated through the Benesi-Hildebrand equation.

Based on the calculation of the curve results in Figures 1 and 2, it was known that the association constant between ethylmorphine and monomers acrylamide (AM) and methacrylic acid (MAA) in butanol solvent sequentially was 14.62 M^{-1} and 6.35 M^{-1}. The value of the association constant indicates the magnitude of the interaction strength between ethylmorphine and functional monomers. When the value of the association constant is greater, the interactions that occur are getting more robust, and the formed complexes during prepolymerization process are more stable [21]. The obtained Ka value showed that ethylmorphine has a greater Ka value to acrylamide, indicated that the interactions that occured were stronger and the formed complexes were more stable, compared to methacrylic acid.

2.2. Synthesis of MIP (Molecular Imprinted Polymer) and NIP (Non-Imprinted Polymer)

MIP and NIP sorbents were synthesized using bulk and precipitation polymerization to compare the effectiveness of each produced polymers. Bulk polymerization is the simplest method for synthesizing MIP, followed by grinding and sieving to get the desired particle size. However, this method will form irregular particles size and shape, causing problems in reproducibility. In addition, sifting will also reduce the yield of the synthesized polymers [22]. These disadvantages lead to the need for other polymerization methods that can provide better polymer characteristics. Precipitation polymerization is another simple and easy alternative method [23].

Moreover, uniform microsphere MIP particles can be obtained through precipitation polymerization. The polymer chains can be formed individually in a solution reaction system without overlapping or undergoing a merging because the solvents used were much more than the solvents used in bulk polymerization. When the polymer was formed, microspheres MIP will precipitate from solution [24]. But, the binding sites in the MIP beads prepared by precipitation methods are inside their network, causing a slow mass transfer of target molecule [16].

To synthesize MIP, five components were needed, including template, functional monomer, cross-linker, initiator, and porogenic solvent [25]. Polymers were synthesized in a mol ratio between template: functional monomer:cross-linker of 1:4:20. In studies conducted by Ersoy et al. [26], Tian et al. [27], and Bhawani et al. [28] reported that the mol ratio produced polymers which have the best value of imprinting factor, adsorption capacity, and binding efficiency.

In both polymerization methods, sonication was performed to aid the dissolution process and remove dissolved oxygen which can inhibit the polymerization process. Acrylamide and methacrylic acid were chosen as functional monomers because it can form a non-covalent bond with ethylmorphine, i.e. hydrogen bond [13]. Non-covalent bond is expected to occur because of its ease in the process of imprinting, removing templates and the binding rate [24].

Porogen selection is essential in MIP synthesis because porogen must have the ability to dissolve all components in one polymerization phase. The porogen used in non-covalent polymerization must be able to stabilize the interaction between ethylmorphine and monomer. Generally, low polarity porogen, both non-polar and aprotic polar porogen, can increase the formation of hydrogen bonds, thereby increasing the efficiency of imprinting [22,24]. Butanol was chosen because it can dissolve all components for polymerization and has a reasonably low polarity, so it was expected to produce MIP with good imprinting effect.

EGDMA (ethylene glycol dimethacrylate) was added as a cross-linker to stabilize the functional monomers around the template to form a crosslinked rigid polymer and to control the morphology of the polymer matrix [24]. Besides that, EGDMA can produce polymers with excellent thermal and mechanical stability, has good porosity, and easy to polymerize [13].

In this study, benzoyl peroxide (BPO) was used as the initiator of the polymerization reaction. BPO is a thermal radical initiator which can form radicals when exposed to heat. BPO will decompose to form benzoyloxy radicals [29]. Then, these free radicals will attack the vinyl group on the monomer, and the polymerization process will begin [30].

NIP served as a control to observe the specificity of the MIP binding site and to ensure the interactions that occur between the functional groups in the polymer with the analyte were molecular interactions and not non-specific interactions [31,32]. Molecular interactions occur at the recognition site that was formed during the imprinting process, while non-specific interactions were caused by random binding of molecules to the polymer [33].

2.3. Extraction of Template from MIP

Template extraction is an important step in MIP preparation [34]. It was aimed to remove ethylmorphine molecules from MIP and leave specific cavities that complement to ethylmorphine in size, shape, and functional groups [25]. If there were ethylmorphine molecules left in MIP, there would be fewer cavities available for rebinding, thereby reducing their efficiency. Moreover, if the template leaking occurred during the analysis process, an error will develop [34].

Ethylmorphine extraction was carried out using ultrasound-assisted extraction (UAE) method. This method can optimize the performance of the solvent in extracting the template, while also minimizing the volume of the solvent used, time, and the costs involved. Ultrasonic waves are mechanical vibrations produced at frequencies more than 20 kHz causing cavitation effects, which form small bubbles in liquid media and mechanical erosion or breakdown of solid particles. As a result, an increase in local temperature will promote solubility and diffusivity, as well as an increase in pressure that supports the penetration and transportation of template molecules out of the MIP [34].

MIP was prepared in a beaker glass and 50 mL of methanol: acetic acid (9:1) were added. According to European Pharmacopoeia [35], ethylmorphine is soluble in alcohol. Therefore, methanol was chosen to extract ethylmorphine from MIP. In addition, acetic acid was added to disrupt the hydrogen bond between ethylmorphine and monomers, making it easier to extract ethylmorphine [31].

Table 1. Ethylmorphine extraction result from MIP.

Compound	Absorbance (288 nm)
Ethylmorphine 20 mg/L	0,083
MIP-AM 1	-
MIP-AM 2	-
MIP-MAA 1	-
MIP-MAA 2	-

Note : (–) no absorption peak observed; AM= acrylamide, MAA= methacrylic acid; 1= MIP synthesized by bulk polymerization; 2= MIP synthesized by precipitation polymerization

Based on Table 1, no absorption peak observed in the measurement of all MIPs at ethylmorphine maximum wavelength, i.e. 288 nm. These results indicate that there was no longer ethylmorphine bound to MIPs.

2.4. Adsorption Ability Evaluation
Evaluation of adsorption ability was carried out to determine the optimal solvent conditions that template can be adsorbed well by the polymer [36]. The evaluation was done by preparing ethylmorphine 20 mg/L in various solvents, i.e. butanol, acetonitrile, and DMSO. Solvents with different polarity were used to determine the types of interactions that occurred between MIP and templates because, in different solvents, the types of interactions that occur will also be different [18].

5 ml of ethyl morphine solution was added to the vial containing 20 mg of MIP and shaken for 3 hours that rebinding occurred between template and MIP until equilibrium was reached [37]. After that, the absorbance of the filtrate was measured using UV-Vis spectrophotometer. The remaining ethylmorphine in the filtrate was the amount of ethylmorphine that was not adsorbed. Therefore, the amount of ethylmorphine adsorbed was calculated by the difference between the initial concentration of ethylmorphine and the concentration of free ethylmorphine in the filtrate [15]. The adsorption ability data is shown in Figure 3 until Figure 6.
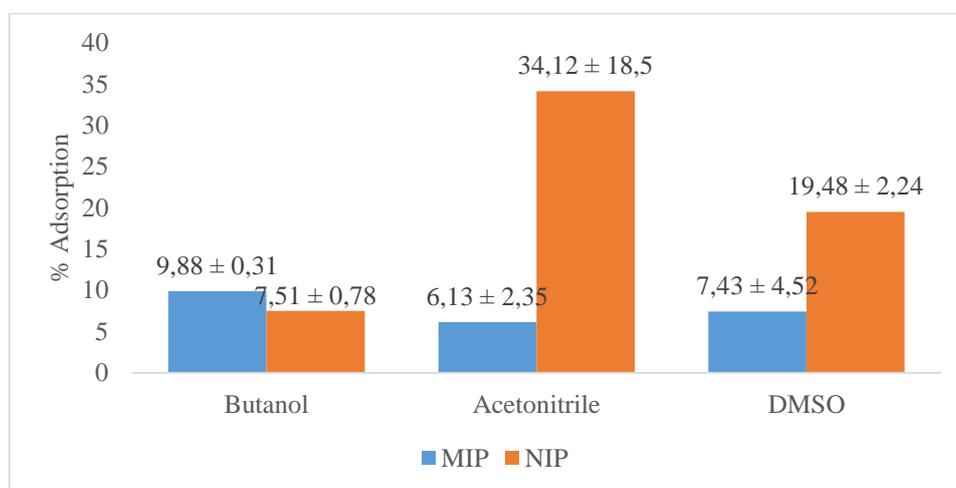
Figure 3. Adsorption ability of AM polymer by bulk polymerization method (AM 1)
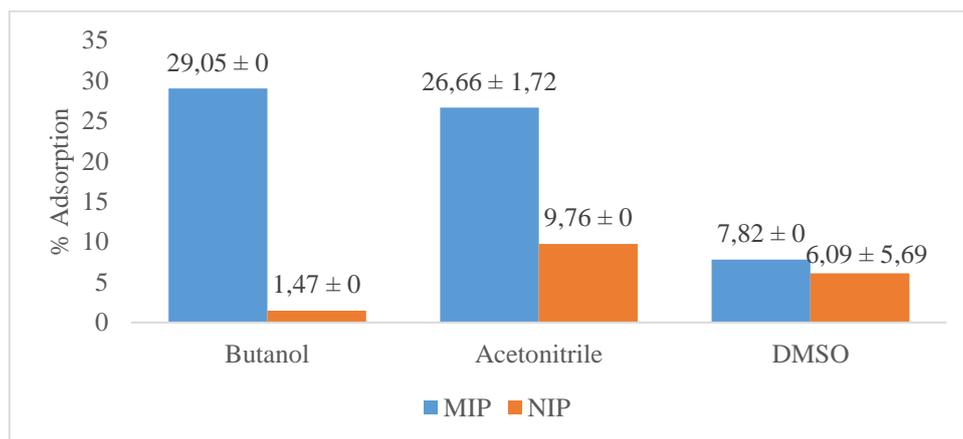
Figure 4. Adsorption ability of AM polymer by precipitation polymerization method (AM 2)
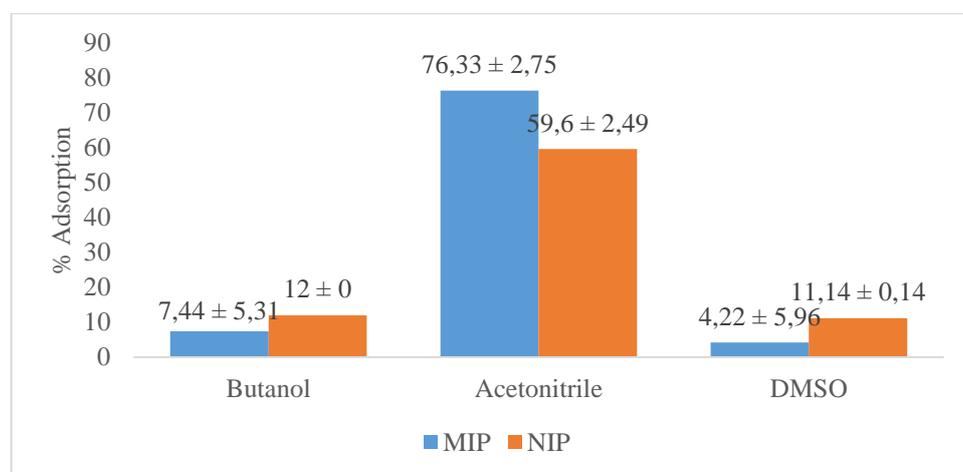
Figure 5. Adsorption ability of MAA polymer by bulk polymerization method (MAA 1)
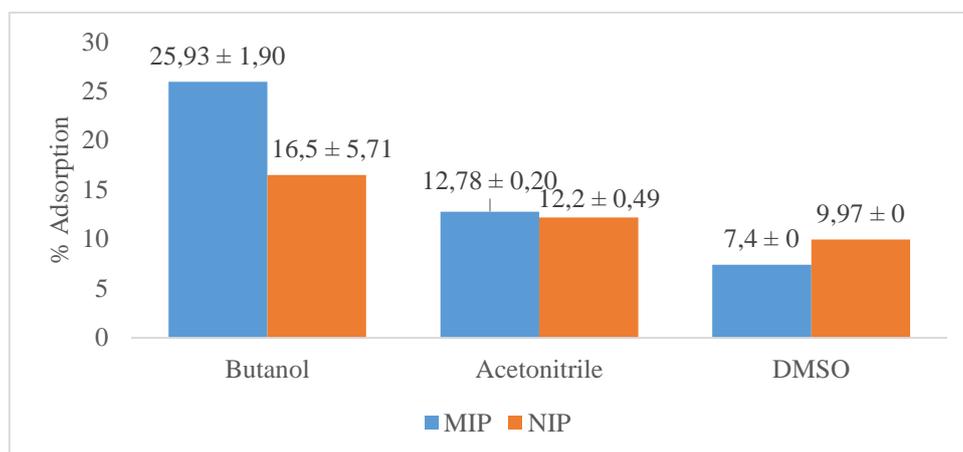
Figure 6. Adsorption ability of MAA polymer by precipitation polymerization method (MAA 2)

Based on Figure 3, Figure 4 and Figure 6, it can be known the adsorption ability of MIP-AM 1, MIP-AM 2, and MIP-MAA 2 showed the most optimal results in the butanol solvent, which was the solvent used during polymer synthesis. Meanwhile, based on Figure 5, it can be seen the adsorption ability of MIP-MAA 1 showed the most optimal results in acetonitrile solvent. The different condition of the solvent required for the MIP-MAA 1 and MIP-MAA 2 for rebinding process was due to the different pore

shapes resulting from the two synthesis methods (bulk and precipitation polymerization). Therefore the swelling ability of each polymer in the different solvent will also be changed [21]. In acetonitrile, MIP-MAA 1 has a higher affinity for ethylmorphine than in other solvents, as well as MIP-AM 1, MIP-AM 2, and MIP-MAA 2 in butanol. In other words, acetonitrile and butanol did not interfere with the interactions that occurred between ethylmorphine and the binding site of MIP (rebinding process) [15,38].

Form all figure of absorption ability, MP-AM 2 made by precipitation polymerization has the highest differences with its NP compared to bulk. The result linear with another result that the uniform microsphere MIP particles can be obtained through precipitation polymerization, and this caused higher adsorption ability [24].

2.5. Adsorption Capacity Evaluation

This evaluation aimed to find out the number of the template (analytes) that can be bound or adsorbed by MIP. The more analytes that can be bound by MIP, the better the binding capacity is [39]. Adsorption capacity can be known by using Freundlich isotherm which describes adsorption in heterogeneous surfaces [36] with the correlation between the equilibrium of analytes that bound to the polymer (sorbent) (B) and the number of free analytes (F) in the following equation [40]:

$$Log\ B = log\ a + m\ log\ F$$

The value of a is the affinity of the polymer when the value of a is higher; it indicates the better polymer capacity in bonding to the analytes. At the same time, m is the homogeneity of the polymer. If the value of m close to 0, it indicates that the system is non-homogeneous, whereas if the value approaches 1 indicates that the system is homogenous [41]. Table 2 shows the value of adsorption capacity of each MIP-AM, MIP-MAA and NIP. The result is MIP-AM 2 has the best affinity value, 3.2×10^{-3} with the homogeneity value -0.77, which indicate that the system is non-homogenous (appropriate with Freundlich isotherm).

Table 2. Adsorption capacity of AM and MAA butanol bulk and precipitation method (n=3).

Value	Bulk polymer				Precipitation polymer			
	MIP-AM 1	NIP-AM 1	MIP-MAA 1	NIP-MAA 1	MIP-AM 2	NIP-AM 2	MIP-MAA 2	NIP-MAA 2
m	3.30	-2.03	1.99	2.84	-0.77	2.30	1.23	-0,10
a (mg/g)	2.8×10^{-6}	2.3×10^{-4}	4.7×10^{-6}	2×10^{-7}	3.2×10^{-3}	1.2×10^{-3}	4.5×10^{-4}	4.6×10^{-6}
R^2	0.965	0.684	0.728	0.927	0.995	0.974	0.857	0.219

2.6. MIP Selectivity Evaluation

Determination of MIP selectivity is to verify that MIP can selectively recognize template molecules (ethylmorphine). The evaluation was done by comparing the ability of MIP to bind ethylmorphine and its analogues: codeine and hydromorphone HCl [42]. The values that used to determine the selectivity of MIP are distribution coefficient (K_D) and imprinting factor (IF) values shown in Table 3 and Table 4.

Table 3. Selectivity of AM and MAA butanol polymer made by bulk polymerization (n=3).

Analyte		Ethylmorphine	Hydromorphone HCl	Codeine
$K_{D\ (ml/g)}$	MIP-AM 1	27.40 ± 0.95	75.79 ± 5.1	103.91 ± 1.83
	NIP-AM 1	20.31 ± 2.27	103.91 ± 2.52	2.12 ± 3
Imprinting Factor		1.35	0.73	0.66
$K_{D\ (ml/g)}$	MIP-MAA 1	882.35 ± 0	1300.05 ± 377.42	89.43 ± 24.10
	NIP-MAA 1	350.49 ± 24.26	72.66 ± 7.19	69.24 ± 26.08
Imprinting Factor		2.52	17.89	1.29

Table 4. Selectivity of AM and MAA butanol polymer made by precipitation polymerization (n=3).

Analyte		Ethylmorphine	Hydromorphone HCl	Codeine
$K_{D\ (ml/g)}$	MIP-AM 2	102.35 ± 0	223.97 ± 10.17	14.92 ± 0.28
	NIP-AM 2	3.73 ± 0	216.78 ± 0	13.18 ± 1.09
Imprinting Factor		27.43	1.03	1.13
$K_{D\ (ml/g)}$	MIP-MAA 2	66.40 ± 37.24	110.36 ± 20.23	31.43 ± 10.93
	NIP-MAA 2	50.10 ± 20.53	128.83 ± 8.42	57.97 ± 10.44
Imprinting Factor		1.32	0.86	0.54

The results showed that all MIP has a bigger distribution coefficient toward hydromorphone HCl. It can happen because hydromorphone HCl is a form of salt from hydromorphone so that when it dissolved in water, it will separate into basic form (hydromorphone). Its acid (HCl), hence the form that will bind to MIP is hydromorphone which has more straightforward molecular shape (see Figure 7). Ethylmorphine can bind selectively to MIP-MAA2 with the value of imprinting factor is 0.86 and has distribution coefficient of 223.97 ml/g, which is higher than its NIP 216.78 ml/g, but still lower than MIP-AM 2. From imprinting factor value that represents selectivity, MIP-AM2 has the higher IF value and selective against hydromorphone HCl and Codeine. The selectivity result showed MIP-AM2 could distinguish ethylmorphine when other compound having similar structure also exist.
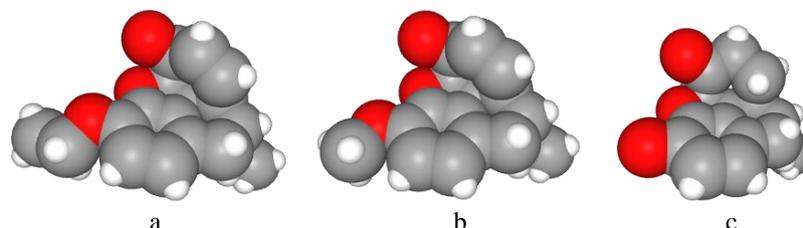
Figure 7. Three dimensional structure of (a) ethylmorphine; (b) codeine and (c) hydromorphone HCl (NCBI, 2019)

2.7. Characterization of Ethylmorphine-Imprinted Polymer

The FTIR spectrums of MIP-AM and MIP-MAA are presented in Table 5-8. Characterization using FTIR instrument aims to confirm that polymer (sorbent) has been successfully polymerized [43] by identifying the functional groups [44]. The complete polymerization process can be characterized by seeing the absence of doublet peak (vinyl group, H2C=CH-) at the wavenumber 995-985 cm^{-1} and 915-905 cm^{-1} and a peak at 1638-1648 cm^{-1}.

Table 5. FTIR Result of MIP-MAA1 dan NIP-MAA1 by Bulk Polymerization.

Wavenumbers (cm^{-1})			Functional Group
MIP-MMA1 before template extraction	MIP-MMA1 after template extraction	NIP-MAA1	
3562,52	3564,45	3564,45	O-H *stretching*
2989,66	2991,59	2989,66	C-H *stretching*
2318,44	2341,58	2341,58	N-H *stretching*
1732,08	1732,08	1732,08	C=O *stretching*
1633,71	1645,28	1635,64	C=C *stretching*
1392,61	1394,53	1386,82	C-H *bending*
1267,23	1265,30	1261,45	C-O *stretching*
1161,15	1166,93	1166,93	C-O *stretching*

Table 6. FTIR Result of MIP-AM1 dan NIP-AM1 by Bulk Polymerization.

Wavenumbers (cm^{-1})			Functional Group
MIP-AM1 before template extraction	MIP-AM1 after template extraction	NIP-AM 1	
3599,17	3606,89	3595,31	N-H *stretching*
2966,87	2966,87	2987,74	C-H *stretching*
1734,01	1732,08	1732,08	C=O *stretching*
1452,4	1456,26	1454,33	C-H *bending*
1388,75	1386,82	1392,61	C-H *bending*
1289,16	1269,16	1269,16	C-N *stretching*
1174,65	1172,72	1165	C-O *stretching*

Table 7. FTIR Result of MIP-MAA2 dan NIP-MAA2 by Precipitation Polymerization.

Wavenumbers (cm^{-1})			Functional Group
MIP-MMA2 before template extraction	MIP-MMA2 after template extraction	NIP-MAA2	
3446,79	3444,87	3444,87	O-H *stretching*
2985,81	2985,81	2985,81	C-H *stretching*
1728,22	1732,08	1732,08	C=O *stretching*
1635,64	1635,64	1635,64	C=C *stretching*
1386,82	1386,82	1386,82	C-H *bending*
1259,52	1259,52	1259,52	C-O *stretching*
1157,28	1161,15	1161,15	C-O *stretching*
954,76	960,55	960,55	C=C *stretching*
879,54	879,54	879,54	C=C *stretching*

Table 8. FTIR Result of MIP-AM2 dan NIP-AM2 by Precipitation Polymerization.

Wavenumbers (cm^{-1})			Functional Group
MIP-AM2 before template extraction	MIP-AM2 after template extraction	NIP-AM 2	
3442,94	3442,94	3444,87	N-H *stretching*
2956,87	2956,87	2956,87	C-H *stretching*
1732,08	1728,22	1728,22	C=O *strecthing*
1454,33	1454,33	1456,26	C-H *bending*
1386,82	1386,82	1386,82	C-H *bending*
1259,52	1259,52	1259,52	C-N *stretching*
1157,29	1159,22	1159,22	C-O *stretching*
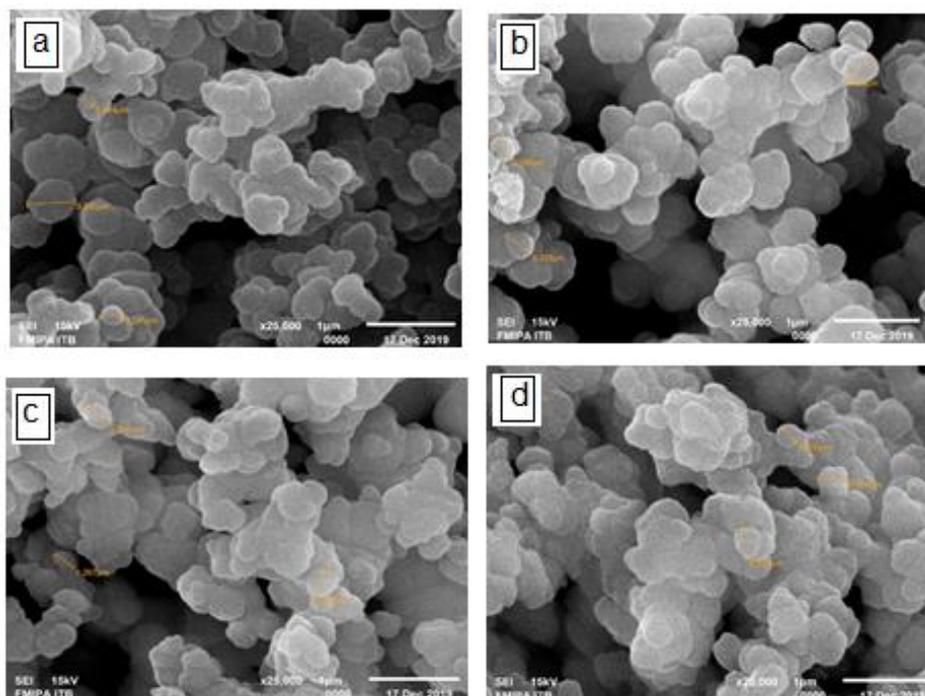
Figure 12. SEM images of (a) MIP-AM 2; (b) NIP-AM 2; (c) MIP-MAA 2 and (d) NIP-MAA 2

Besides MIP-AM 1, each of FTIR spectrum indicates a vinyl group. Because of that, MIP-AM 2, MIP-MAA 1, and MIP-MAA 2 may have a low degree of polymerization.

The morphologies, particle size, geometry and the surfaces of polymer (sorbent) can be characterized using SEM [15]. The result from SEM images of MIP-AM 2 and MIP-MAA 2 can be seen in Figure 12. Both of MIP-MAA 2 and MIP-AM 2 had spheric form

3. Conclusion

The molecularly imprinted polymer (MIP) of ethylmorphine with AM as the functional monomer in butanol using the precipitation polymerization method had better analytical performances than MIP-MAA 2. The affinity value was 0.072 mg/g with the homogeneity value of -0.77. It can recognize ethylmorphine selectively with the value of IF (imprinting factor) 27.43. In addition, physical characterization showed that MIP-AM 2 may have a low degree of polymerization due to the presence of vinyl peaks and had a smaller particle size than its NIP. From the study, MIP-AM made by precipitation polymerization could be used to extract ethylmorphine on solid-phase extraction to analyze ethyl morphine in the biological fluid.

4. Experimental

4.1. Materials

Acrylamide (Sigma Aldrich), methacrylic acid (Sigma Aldrich), acetic acid (Merck), acetonitrile (Fisher Chemical), benzoyl peroxide (Merck), butanol (Merck), dimethyl sulfoxide (DMSO) (Merck), ethylene glycol dimethacrylate (EGDMA) (Sigma Aldrich), ethylmorphine (P.N.F. Nakula Farmasi), hydromorphone HCl, codeine (Kimia Farma), potassium bromide (Merck), and methanol HPLC grade (Merck). All materials are in pro analysis grade. The instrument that used in this study were *Fourier Transform Infrared* (FTIR) (IRPrestige-21 Shimadzu), *Scanning Electron Microscope* (SEM) (JEOL JSM 6510-LA), UV-Vis spectrophotometer (Analytik Jena specord 200), agitator (IKA® HS 260 basic), centrifugation devices (Yenaco and Hettich Zentrifugen EBA 20), mesh 60 sieves, oven (Memmert), digital scales (Ohaus Pioneer), ultrasonic (NEY 19H), water bath (Memmert), and glass tools commonly used in chemical analysis laboratory.

4.2. Determination of Monomer-Template Association Constants Using UV-Vis Spectrophotometers

2.5 mL of ethylmorphine solution (20 mg/L) in butanol was measured using UV-Vis spectrophotometer. Acrylamide solution of 10.000 mg/L or methacrylic acid solution of 13.000 mg/L was added into the solution gradually from 10 μl, 20 μl, 30 μl, 40 μl, 50 μl, to 100 μl. The absorbance of the solution was measured at each addition of the monomer solution. Addition of

and smaller particle size than its NIP with the average value of 0,31 ± 0,21 μm and 0.28 ± 0.05 μm respectively. The smaller particle of MIP compared to NIP showed higher selectivity toward the similar compound. MIP-AM 2 with smaller particle than MIP-MAA had higher imprinting factor, which is 27.43 based on Table 4. This is indicated The small particle size can make the ability of polymer in adsorb become larger [18].

monomer solution was stopped when there was no significant increase in the absorbance value [45].

A graph between $1/\Delta Y$ and $1/[G]$ was made to determine the value of the association constant through the Benesi-Hildebrand equation:

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

where ΔY is the change in absorbance, $Y\Delta HG$ is the change in absorbance at the end point of the titration, K_a is the association constant, and $[G]$ is the monomer concentration added [20].

4.3. Synthesis of MIP (Molecular Imprinted Polymer) and NIP (Non Imprinted Polymer) Using Bulk Polymerization

Polymers synthesis were carried out using two functional monomers, i.e. acrylamide and methacrylic acid. Ethylmorphine (1 mmol) as a template and acrylamide or methacrylic acid (4 mmol) were dissolved in 3.5 ml of butanol in a closed vial and sonicated for 5 minutes. After completely dissolved, EGDMA (20 mmol) as cross-linker was added and sonicated for 10 minutes. 250 mg of benzoyl peroxide as an initiator was added and sonicated for 20 minutes. Vials are sealed with parafilm after purged with N_2 and heated in a water bath for 18 hours at 80°C. Then, the formed polymers were crushed and sieved using mesh 60, washed using 50 mL of methanol: water (1:1) mixture, and dried in an oven at 70°C for 18 hours. Non-imprinted Polymer (NIP) was also synthesized simultaneously under the same condition without the addition of ethylmorphine molecules (template) [15,46].

4.4. Synthesis of MIP (Molecular Imprinted Polymer) and NIP (Non-Imprinted Polymer) Using Precipitation Polymerization

Ethylmorphine (1 mmol) and acrylamide or methacrylic acid (4 mmol) were dissolved in 350 mL of butanol in a closed vial and sonicated for 5 minutes. EGDMA (20 mmol) was added and sonicated for 10 minutes. Benzoyl peroxide 550 mg was added and sonicated for 20 minutes. Vials are sealed with parafilm after purged with N_2 and heated in a water bath shaker for 18 hours at 80°C. Then, the formed polymers were washed using 50 mL of methanol: water (1:1) mixture and dried in an oven at 60°C for 18 hours. Non-imprinted Polymer (NIP) was also

prepared simultaneously under the same condition without the addition of ethylmorphine molecules [15,46].

4.5. Extraction of Template from MIP

Ethylmorphine template was extracted from MIP using a sonicator. MIP was prepared in a beaker glass and 50 mL of methanol: acetic acid (9:1) mixture was added. Beaker glass was covered with a plastic wrap and sonicated for 3 hours. Then, MIP was washed with methanol and water and dried in an oven at 55°C for 18 hours [47].

Extraction results were monitored using UV-Vis spectrophotometer to ensure the ethylmorphine was extracted entirely. 20 mg of extracted MIP was added by 5 mL of methanol, triplicate. Then, the mixture was shaken for 3 hours. The absorbance of the filtrate was measured. The extraction process is complete when the monitoring results showed there was no longer ethylmorphine remaining in the polymers [45].

4.6. Adsorption Ability Evaluation

Ethylmorphine solutions of 20 mg/L were made in a variety of solvents, i.e. butanol, acetonitrile, and DMSO. 5 ml of the ethylmorphine solution was added into the vial containing 20 mg of MIP, triplicate. 10 ml of solvent without ethylmorphine was added into 40 mg of MIP as a blank. Then the agitation was carried out for 3 hours. After that, the filtrate was taken, and the absorbance was measured with UV-Vis spectrophotometer. The amount of ethylmorphine adsorbed was calculated based on the difference between the initial concentration of ethylmorphine and the concentration of free ethylmorphine in the filtrate. The evaluation was carried out on NIP by the same procedure [15].

4.7. Adsorption Capacity Evaluation

A 5 ml of selected solvent from binding ability evaluation was prepared by varying the concentration of ethylmorphine solution of 15, 20, 25, 30 and 35 mg/L. Then it was added to 20 mg of MIP, triplicate for each concentration. The mixture was shaken using a shaker at 120 rpm for 3 hrs. The filtrate was measured by using UV-Vis spectrophotometer. NIP was also evaluated with the same procedure. The results of the adsorption capacity can be determined by using the Freundlich isotherm adsorption curve [23,40].

4.8. MIP Selectivity Evaluation

Determination of MIP selectivity can be known by calculating the distribution coefficient (K_D) of ethylmorphine, hydromorphone HCl, and codeine solutions. 5 ml from each solution was added to 20 mg of MIP, triplicate. The mixture was shaken using a shaker at 120 rpm for 3 hrs. The filtrate was measured by using UV-Vis spectrophotometer. NIP was also evaluated with the same procedure. Calculate the imprinting factor by using the following equations:

$$K_D = \frac{(Ci - Cf)V}{Cf\ m} \qquad IF = \frac{K_D\ MIP}{K_D\ NIP}$$

K_D is distribution coefficient, Ci is the initial concentration (before adsorption), Cf is the final concentration (after adsorption), V is the volume of solution, and m is mass of the polymer (sorbent); while IF is imprinting factor [46,48].

4.9. Characterization of Ethylmorphine-Imprinted Polymer

Physical characterization of MIP and NIP samples was using FTIR spectroscopy (IRPrestige-21, Shimadzu) by ground adpressed the samples into KBr plates than analyze it between 400 and 4000 cm^{-1}. The surface morphology of samples was analyzed by using SEM [23,49].

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