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Original Article

Comparison of Bulk and Precipitation Polymerization Method of Synthesis Molecular Imprinted Solid Phase Extraction for Atenolol using Methacrylic Acid

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ABSTRACT

Objective: Atenolol is one of beta-blocker are prohibited as doping based on World Anti-Doping Agency (WADA). The purpose of this study was to the synthesis of molecular imprinted polymer (MIP) for extraction of atenolol from the sample. **Method:** This research compared the two of the method, bulk and precipitation polymerization. The MIP was successfully prepared from methacrylic acid as a functional monomer, ethyleneglycoldimethacrylate as a crosslinker, benzoyl peroxide as an initiator, butanol as a porogenic solvent with atenolol as a template molecule. **Result:** The result showed that the bulk polymerization method produces sorbents that have good adsorption capacity and small particle compare to the precipitation polymerization. Both methods were selective for atenolol. **Conclusion:** Generally, the MIP solid phase extraction is an alternative method for extraction atenolol from the sample.

Key words: Atenolol, Molecular Imprinted Polymer, Solid Phase Extraction.

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INTRODUCTION

Doping refers to prohibited drug, substance or material that used by athlete to improve their performance.[1] Various types of doping are used for reduce anxiety, increase muscle mass, reduce weight or to cover another drug during health check. Doping can cause harmful effect for human include myocardial infarction, hyperlipidemia, hypertension, thrombosis, heart failure and sudden death.[2] Atenolol is one of beta-blocker group that usually used as doping by athlete to reduce anxiety, tremor and low heart rate.[3] Doping analysis can be determined through metabolite or specimens examination. It requires sensitive instruments with pure samples and completely separated from the matrices. Numerous analytical method are used to determine atenolol such as High Performance Liquid Chromatography (HPLC).[4] Gas Chromatography-Mass Spectrometry (GC-MS)[5] and diffuse reflectance spectroscopy.[6] Recently, solid phase extraction (SPE) based on molecular imprinting polymer (MIP) has been developed as a separation technique is expected to be low cost, practical and applicable and has a high recovery percentage. Sorbents with molecular imprinting techniques have a recognizable binding sites that can bind with specific drug targets, thereby being able to separate drugs with complex matrices. Synthesis of molecular imprinted polymer consist of monomer, crosslinker, inisiator and porogen. Monomer must be able to interact with the template form a specific complex donor-receptor in polymerization. Methacrylic acid is an universal monomer that usually used in MIP. This monomer increase imprinting effect through dimerization reaction.[7] Synthesis of MIP-SPE atenolol based on non-covalent bonding using methacrylic acid result the good sorbent with acetonitril or mix acetonitril as a porogen.[8-10] Porogen that usually used in non-covalent bonding MIP is a solvent that has low dielectric constanta, tend to non-polar solvent, because polar solvent can interfere the hydrogen form. In this research, MIP-SPE atenolol was synthesized using methacrylic acid as functional monomer and butanol as a porogen by bulk and precipitation polymerization method.

MATERIALS AND METHODS

Materials

All of material used is analytical grade. Atenolol, metoprolol tartrate hydrochloride and propanolol hydrochloride were obtained from Tokyo Chemical Industry. Methacrylic acid and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma Aldrich. Acetone, alcohol 95% and acetic acid 96% were purchased from Brataco. Acetonitrile and methanol were obtained from Fischer Scientific. Butanol, benzoyl peroxide and potassium bromide were purchased from Merck. The absorbance measurement was recorded by UV–visible spectrophotometer (Analytical Jena Specord 200 using a 1.0 cm quartz cell). Identification of functional group was analyzed by Fourier Transform Infrared (FTIR) IR (Prestige-21 Shimadzu).

Methods

Determination of the Association Constant of Monomer-Template Complex using UV Titration Method

Determination of the association constant can describes the interaction of monomer and template. Stock solution of atenolol in butanol was prepared in 2 x 10^{-5} M and methacrylic acid was 5 x 10^{-3} M. Atenolol solution was measured by UV-visible spectrophotometer then methacrylic acid was added gradually until the absorbance tend to stable. The association constant was calculated by Benesi-Hildebrand equation.[11]

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

ΔY = Absorbance; = Absorbance of HG complex – Absorbance of H; Ka= Association constant; [G]= Consentration of *guest* (monomer).

Synthesis of Molecular Imprinted Polymer by Precipitation Polymerization Method

Methacrylic acid (4 mmol) as a monomer was added into atenolol solution in butanol (1 mmol) as a template in 350 ml solvent. The mixture was sonicated for 5 mins in a closed vial. Then, 20 mmol EGDMA as cross-linker was added and continued to sonicate for 20 mins. 1 mmol benzoyl peroxide was added into solution as initiator, sonicated for 5 mins, then oven at 70°C for 2 hrs. The solution moves to water bath shaker at 70°C for 18 hrs. Subsequently, the solution was centrifuged and the precipitation was washed by methanol and water. The polymer was dried in oven at 60°C for 18 hrs. To verify the MIP results, the Non Imprinted Polymer (NIP) was also synthesized using this steps but without template.

Synthesis of Molecular Imprinted Polymer by Bulk Polymerization Method

Atenolol was dissolved in butanol (1 mmol) in closed vial then 4 mmol methacrylic acid was added and sonicated for 5 mins. EGDMA (20 mmol) as cross linker was added and continued to sonicate for 20 mind. Then, benzoyl peroxide (1 mmol) as an initiator was added to the solution. The solution was moved to oven at 70°C for 2 hrs and then to the water bath shaker at 70°C for 18 hrs. The polymer was mashed and filtered using a mesh size of 60. Afterward, the polymer was rinsed with methanol and water then dried in oven at 70°C for 18 hrs. The Non Imprinted Polymer (NIP) was also synthesized using this steps but without template.

Adsorption Capability Evaluation

Evaluation of adsorption capability was carried out in methanol, acetonitrile, acetonitrile: methanol (1: 1) and acetonitrile: methanol (1: 9). Sorbent of MIP (20 mg) was dissolved in atenolol solution of 5 ppm (in different solvent) and allowed to stand for 24 hrs. Filtrate from the mixture was measured by UV-Vis Spectrometry. The adsorption capability was calculated by the difference between the initial atenolol concentration and the free atenolol concentration in the filtrate. The NIP sorbent was also evaluated by the same procedure.

Adsorption Capacity Evaluation

Evaluation of adsorption capacity was carried out by varying the concentration of atenolol solution of 1, 2.5, 5, 7.5 and 10 ppm. A 5 ml atenolol solution from each concentration was added into 20 mg of MIP sorbent. The mixture was shake and allowed to stand for 24 hrs. The filtrate was measured by UV-Vis spectrometry. NIP sorbent was also evaluated by the same procedure. The adsorption capacity was calculated by using Freundlich isotherm adsorption curve.[12-13]

MIP Selectivity Evaluation

Evaluation of MIP selectivity was determined by calculating the coefficient of distribution of atenolol, metoprolol and propranolol solution at 5 ppm. A 5 ml of each solution was added into 20 mg of MIP sorbent. The mixture was shake and allowed to stand for 24 hrs. The filtrate was measured by UV-Vis spectrometry. NIP sorbent was also evaluated by the same procedure. The distribution coefficient was calculated by the following equation:[14]

$$K_D = \frac{C_p}{C_s}$$

K_D is distribution coefficient, Cp is concentration of substrate in polymer (mol/g) and Cs is concentration of substrate in solution (mol/g). The ratio of K_D MIP and K_D NIP was calculated as imprinting factor value.[12]

RESULT

Determination of the Association Constant of Monomer-Template Complex using UV Titration Method

Interaction of monomer-template can be analyzed by determination of the association constant. Association constant was calculated based on slope and intercept on Bennesi-Hildebrand equation. Based on Figure 1, Ka of atenolol and methacrylic acid was 9.24 x 10^2 M^{-1}.

Comparison of Physical Characterization of MIP by Precipitation and Bulk Polymerization Method

Physical characterization of MIP was analyzed by using FTIR and SEM. FTIR to describe the functional group on the compound and SEM (Scanning Electron Microscope) to describe the morphology of the polymer. Table 1 and Table 2 show the FTIR analysis of MIP and NIP sorbent. The SEM analysis show in Figure 2 and Figure 3.

Adsorption Capability Evaluation

The results of adsorption capability of the MIP sorbent are shown in Figure 4 for the bulk polymerization and Figure 5 for precipitation polymerization.

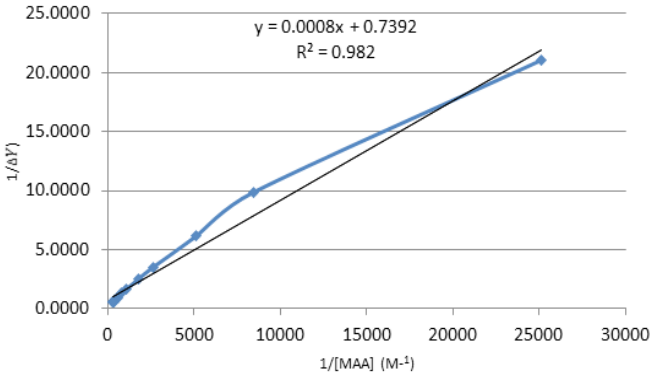

Figure 1: Graph of association constant of atenolol and methacrylic acid.

Table 1: FTIR analysis of MIP and NIP sorbent by bulk polymerization.

Wave number (cm^{-1})			Functional Group
Sorbent of MIP before extraction	Sorbent of MIP after extraction	Sorbent of NIP	
3580.91	3563.55	3594.41	-OH stretching
3461.32	-	-	N-H stretching
2347.71	2974.29	2974.29	C-H stretching
1735.00	1734.04	1733.07	C=O stretching
1635.00	1633.74	1634.70	C=C stretching
1407.85	1466.89	1467.86	CH_2 bending
1100.20	1160.20	1162.13	C-O stretching

Table 2: FTIR analysis of MIP and NIP sorbent by precipitation polymerization.

Wave number (cm^{-1})			Functional Group
Sorbent of MIP before extraction	Sorbent of MIP after extraction	Sorbent of NIP	
3590.55	3582.84	3571.26	-OH stretching
3464.21	-	-	N-H stretching
2980.78	2970.43	2971.39	C-H stretching
1731.14	1729.21	1731.14	C=O stretching
1635.66	1633.74	1635.66	C=C stretching
1463.03	1464.00	1464.00	CH_2 bending
1157.31	1157.31	1156.34	C-O stretching

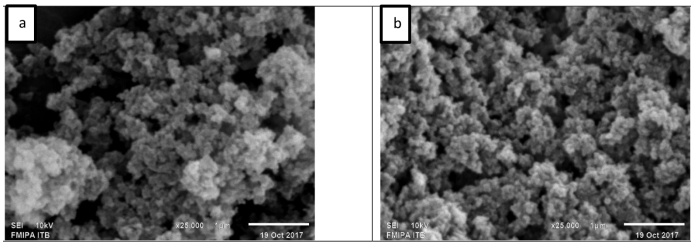

Figure 2: SEM analysis of MIP (a) and NIP (b) by bulk polymerization.

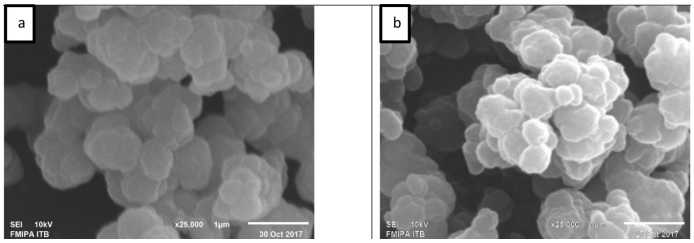

Figure 3: SEM analysis of MIP (a) and NIP (b) by precipitation polymerization.

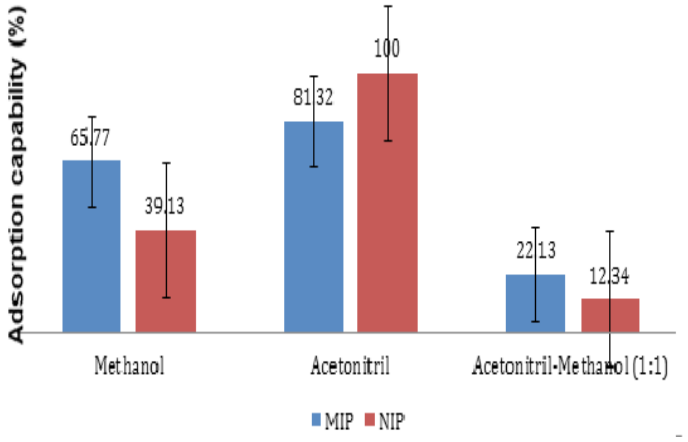

Figure 4a: Graph of adsorption capability of MIP and NIP sorbent by bulk polymerization (n=3).

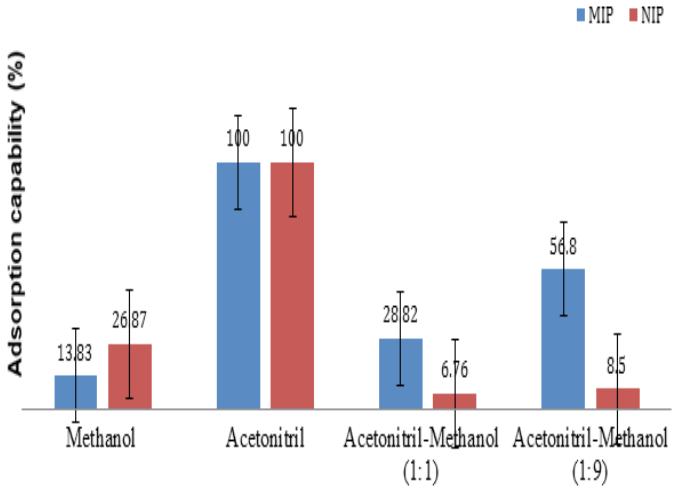

Figure 4b: Graph of adsorption capability of MIP and NIP sorbent by precipitation polymerization (n=3, mean±SD)

Table 3: The adsorption capacity of MIP and NIP sorbent of bulk and precipitation polymerization.

Value	Bulk polymerization		Precipitation polymerization	
	MIP	NIP	MIP	NIP
m	0.893	0.757	2.567	1.197
a (mg/g)	7.804	4.819	2.950	5.740
r	0.81	0.99	0.83	0.71

Table 4: Selectivity of MIP and NIP Sorbent by bulk polymerization (n=3).

Analyte		Atenolol	Propranolol	Metoprolol
KD	MIP	486.97±2.0	101.42±0.5	195.04±0.5
	NIP	169.80±2.1	83.19±0.6	530.73±0.7
Imprinting Factor		2.87±0.2	1.22±0.5	0.37±0.4

Adsorption Capacity Evaluation

The adsorption capacity was calculated by using Freundlich isotherm adsorption curve. The result show in Table 3.

MIP SelectivityEvaluation

Evaluation of MIP selectivity was determined by calculating the coefficient of distribution and imprinting factor. Table 4 show the selectivity of MIP and NIP Sorbent by bulk polymerization and Table 5 show the selectivity of MIP and NIP Sorbent by precipitation polymerization.

DISCUSSION

Interaction of methacrylic acid as a monomer and atenolol as a template can be analyzed by determination of the association constant. Generally, the better and stronger interactions that occur, the better of the imprinting effect and the more stable of the complex during polymerization.[15] Therefore, the interaction of monomer-template must be tested by non-covalent imprinting stoichiometry study.[16] In this study, the interaction was determined based on association constant value (Ka). If the Ka is around 10^3 M^{-1}, the complex is stable and the binding site has a good performance with recovery value more than 90%.[17] Based on Figure 1,

Table 5: Selectivity of MIP and NIP Sorbent by precipitation polymerization (n=3).

Analyte		Atenolol	Propranolol	Metoprolol
KD	MIP	325.43±3.1	44.51±0.4	207.62±0.2
	NIP	78.22±2.1	68.79±0.5	191.30±0.3
Imprinting Factor		4.16±2.1	0.65±0.3	1.09±0.2

Ka of atenolol and methacrylic acid was 9.24 x 10^2 M^{-1}. This value is close to the expected value, so it can be predicted that the interaction of monomer and template are strong.

FTIR analysis can confirm the success of co-polymerization step.[18] The –OH stretching band in Table 1 and Table 2 related to the carboxylic group (-COOH) in the monomer of methacrylic acid. The –CH and CH_2 stretching related to the methylene group in methacrylic acid and EGDMA. The strong intensity of wavenumber around 1700 cm^{-1} show the functional group of C=O from EGDMA, methacrilic acid and benzoyl peroxide. The absence of twin peaks in the area of the wave number 900-1000 cm^{-1} indicates the absence of a vinyl group which means the polymerization process is complete.

Scanning Electrone Microscope was used to describe the geometry, particle size and surface of the polymer. Based on Figure 2 and Figure 3, the surface of NIP is more smooth and homogeneous compare to MIP which has a coarser and large cavity as a results from template extraction. It indicates that the specific cavity is formed. Polymer from precipitation polymerization method have a surface with relatively larger particles compare to the bulk polymerization.

The sorbent that has been synthesized will absorb the analyte. In this process needed to find the optimum condition to be able to absorb analytes to the fullest. One of the important thing in the test of sorbent-analyte binding is polymerization technique and the use of solvents during binding testing.[19] In this research, the solvent variations were tested for the adsorption ability of MIP and NIP. Figure 4 show th**a**t the MIP sorbent by bulk polymerization has the optimum adsorption in methanol and acetonitrile at 65.77 and 81.32%, respectively. In acetonitrile, NIP has the higher adsorption compare to the MIP. Atenolol more easily dissolve in methanol than acetonitrile. In this condition, methanol can bring the atenolol to the binding site that spread in MIP. In the other hand, methanol also can bring back atenolol which has less interaction at the binding site. Acetonitrile has a lower interaction with atenolol. When it reacts with acetonitrile, atenolol will dissolve first then bring to the binding site. When interaction of analyte-MIP is stronger than analyte-solvent, analyte will retain in MIP.[20] Therefore, adsorption capability of acetonitrile is haigher than methanol. However, in acetonitril the capability of MIP is lower than NIP, so that methanol was choosen as a solvent because has a higher adsorption capability of MIP. In the precipitation polymerization, acetonitrile, acetonitril:methanol (1:1) and acetonitril:methanol (1:9) has a good MIP adsorption. Although the adsorption in acetonitril was 100% for MIP and NIP, acetonitril:methanol (1:9) was choosen as a solvent because in this condition MIP has a better activity than NIP.

The adsorption capacity was determined by using Freundlich isotherm adsorption curve. This system describes the adsorption in heterogen surface.[21] Freundlich isotherm describes the correlation between the equilibrium of the number of analytes bound to the adsorbent (B) and the number of analytes that remain free (F) as the equation:[22]

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

a is the adsorbent affinity and m is a adsorbent homogeneity. If the value of the m is 0, it indicates that the system is non-homogenous, meanwhile if the value of m is 1 the system is homogenous.[23]

In the bulk polymerization, MIP has the m value close to 1 means that MIP is more homogenous than NIP. The value of a describe the adsorbent capacity in absorb the analyte. In bulk polymerization, MIP has able to absorb up to 7.804 mg/g compare to the NIP. It is indicates the binding site of the sorbent is complement with the shape and size of the template. However, this value is still relatively small because the amount of analyte that can be absorbed is small, so so it takes a large amount of sorbent to absorb more analytes. In the precipitation polymerization, MIP less homogenous than the NIP because NIP has the m value close to 1. Besides that, MIP has the less adsorption capacity compare to the NIP. It can be conclude that adsorption capacity of MIP from bulk polymerization is better than from precipitation polymerization.

The selectivity of MIP was carried out by using metoprolol and propranolol, as a same beta blocker group with atenolol. The selectivity was determined by calculating distribution coefficient distribution that describes the number of analytes absorbed to the concentration of analytes in solution.[14] In the bulk and precipitation polymerization, KD of atenolol is higher than others. It indicates that MIP sorbent is selective for atenolol. MIP was synthesized using atenolol as a template so the cavity of the MIP was formed the cavity for the atenolol. Imprinting factor is also calculated to see the ratio between MIP and NIP. It is describes the performance of MIP. Both of method has the higher imprinting factor of atenolol compare to the others and precipitation polymerization has the higher imprinting factor of atenolol than bulk polymerization. It is indicates that selectivity of MIP form precipitation method is better than bulk method, however both of method has the good performance compare to NIP.

CONCLUSION

The sorbent of MIP can be synthesized by using methacrylic acid as a monomer, butanol as a porogen and EGDMA as a crosslinker with ratio 1:4:20. The sorbent of MIP can be synthesized by using both of method, bulk and precipitation polymerization method. The result show that polymer from precipitation polymerization have a surface with relatively larger particles compare to the bulk polymerization. The sorbent from bulk polymerization has the higher adsorption capacity (7.804 mg/g) compare to the polymerization method (2.95 mg/g). Both of method are selective for atenolol but MIP form precipitation method is more selective than bulk method.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SPE: Solid Phase Extraction; **MIP:** Molecular Imprinted Polymer; **NIP:** Non Imprinted Polymer; **EGDMA:** Ethylene Glycol Dimethacrylate; **FTIR:** Fourier Transform Infrared; **SEM:** Scanning Electron Microscope.

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