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STUDY OF THE BINDING ABILITY OF MOLECULAR IMPRINTED SOLID PHASE EXTRACTION FOR GLIBENCLAMIDE BY OPTIMIZING TEMPLATE : MONOMER : CROSSLINKER RATIO

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ABSTRACT

Five molecularly imprinted polymer for glibenclamide was prepared by using glibenclamide as the template, acrylamide as the functional monomer and ethyleneglycoldimethacrylate as a cross linker in different ratios. Binding ability analysis showed that acetonitrile pH 4 is the best solvent for rebinding of the analyte. The imprinting factor (IF) was calculated by comparing the retention of glibenclamide on the imprinted polymer with a comparable non-imprinted polymer. The template : monomer : crosslinker ratio of 1:6:70 resulted in an IF of 6.85. This MIP has the potential for use in SPE for purification and concentration of glibenclamide and with further optimization.

Key words: Acrylamide, Non covalent polymerization, Glibenclamide, Molecular imprinted solid phase extraction.

INTRODUCTION

Solid Phase Extraction (SPE) is a sample preparation method that is widely used in the analysis process because it can reduce the time and amount of solvent extraction, as well as having high recoveries, especially in the preparation of biological samples[1]. Conventional SPE has drawbacks in terms of selectivity so that there is the possibility of other components other than the analyte to be extracted from the sample matrix[2]. Increased selectivity of conventional SPE can be performed using Molecular Imprinting Polymer technique known

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as Molecular Imprinted Solid Phase Extraction (MISPE)[3]. MIPs (Molecular Imprinted Poymers) is a synthetic polymer obtained by copolymerization with crosslinking monomers (cross-linker), in the presence of template[4,5]. After the polymerization process and a template extraction, produced a cavity (cavities) that has the size, shape, and chemical functionality conformation according to the template[6]. MISPE superiority lies in its ability to selectively isolate the specific compound or its structural analog of a complex matrix[3].

Glibenclamide is a second-generation sulfonylurea drugs for the treatment of diabetes mellitus. Glibenclamide is capable of stimulating the release of insulin from pancreatic beta cells and peripheral tissue sensitivity to insulin[7].

Glibenclamide is white crystalline powder, odorless, has a molecular weight of 494 g/mol and pK value of 5.3, is not soluble in water and ether, sparingly soluble in ethanol and methanol, as well as partially soluble in chloroform (Ministry of Health of the Republic of Indonesia, 1995)[8]. Up to now, a glibenclamide-imprinted polymer from acrylamide is not reported for molecular recognition in aqueous solvents. That is why it is very important to prepare MIPs capable of recognition in aqueous solutions because biological recognition is mainly occurring in aqueous solvents.

MIP sorbent selectivity influenced by several factors, one of which is the type and amount of crosslinking agent used in the polymerization process as well as a functional monomer concentration used[6,9]. Suitability amount of cross-linker is necessary to maintain the stability of the cavity and the polymer matrix[6]. Increasing the concentration of the monomers used for the preparation should increase the number of non- covalent interactions during polymerization which would alter the imprinting factor[9]. The use of a number of monomers in the manufacture of MIP-SPE sorbent will affect the ability of adsorption seen from the relative selectivity coefficient/imprinting factor (IF). Relative selectivity coefficient is the ratio of the value of k (distribution coefficient) of polymer MIP with NIP polymers[9]. In this study, an effort to achieve high imprinting factor of glibenclamide molecular imprinting was done by altering the monomer : template : cross-linker ratio during preparation.

EXPERIMENTAL

Materials used in this study were 2-2-azobis-isobutyro-nitrile (AIBN) (Aldrich), acrylamide (Fluka), acetic acid (Merck), ammonia (Merck), ethyleneglycol dimethacrylate (EGDMA) (Aldrich), glibenclamide (USV), potassium bromide (Merck), chloroform

(Merck), and methanol (JT Baker). The method used include polymer synthesis MIP-SPE with bulk polymerization method, extraction of glibenclamide template MIP-SPE, evaluation of binding ability by using batch rebinding experiment, and determination of the bond strength by using FTIR instrument. Synthesis of MIP-SPE polymer was done by Bulk Polymerization Methods. Glibenclamide (templates) and acrylamide monomer dissolved in chloroform, in a test tube sealed and sonicated for 5 min. EGDMA as cross-linker ratio and 2-2-azo bis-iso butyro-nitrile (AIBN) as the initiator was added to the solution. The mixture was sonicated and purge for 40 min. to remove oxygen. Furthermore, a test tube containing the mixture was placed in a water bath of 60ºC for 18 hrs. Polymers formed are crushed, then sieved with 60 mess and washed using methanol. After washing, the polymer was dried in an oven at a temperature of 60ºC for 18 hr. In order to verify the retention of the resulting MIP, non-imprinted polymer (NIP) also prepared in the same way with MIP but without the addition of the template.

Extraction of glibenclamide template MIP-SPE was conducted by Soxhlet followed with sonication. Extraction performed for 24 hrs by using methanol: acetic acid (9:1). After Soxhlet, the polymer then sonicated with methanol pH 4 for 2 hrs. The extraction process is complete when the liquid leaching results in MIP-SPE sorbent no longer contains the template when monitored using high performance liquid chromatography.

Evaluation of the binding ability of the MIP-SPE was done by using the batch method. 20 mg of MIP sorbent was incorporated into 5 mL glibenclamide at concentrations of 5 ppm, then shaken using a shaker for 3 hours at room temperature 120 rpm. After 3 hrs, the filtrate was measured by using a spectrophotometer UV.

Glibenclamide is used to determine the relative selectivity of the MIP sorbent. 20 mg of MIP-SPE sorbent was mixed with 5 mL of glibenclamide and with a concentration of 5 ppm each. Then it was shaken using a shaker for 3 hrs at room temperature 120 rpm, and then filtered. The filtrate was measured by using high performance liquid chromatography. The imprinting factor is calculated using the equation.

$$IF = Kd\ MIP/Kd\ NIP$$

Kd is a coefficient distribution, V = volume, IF = imprinting factor

The possibility of the hydrogen bonding present were determined by Fourier Transform Infra Red (FTIR).

RESULTS AND DISCUSSION

Selection of MIP functional monomer plays an important role. Amine group on glibenclamide expected to be easy to bind the acid or neutral monomer[10-12]. Commonly used monomer is acrylamide. Acrylamide is a chemical compound that has amine and carbonyl functional groups that can bind to the amine and carbonyl group of the template molecules to form hydrogen bonds[13]. The schematic illustration of the molecular imprinting procedure is shown in Fig. 1.

Table 1: Preparation of mips (imprinted) and nips (non-imprinted, no template) with different ratios of template (SDM): Monomer (MAA): Cross-linker (EGDMA)

Polymer	Template : Monomer : Cross-linker
MIP 1	1:6:40
NIP 1	0:6:40
MIP 2	1:6:60
NIP 2	0:6:60
MIP 3	1:6:70
NIP 3	0:6:70
MIP 4	1:4:40
NIP 4	0:4:40
MIP 5	1:15:40
NIP 5	0:15:40

Because glibenclamide has more locations of potential non-covalent interactions, it was hypothesized that using glibenclamide as the template and increasing the monomer concentration used during the preparation should increase the number of non-covalent interactions during polymerization, thus improving the imprinting factor. Using a higher template: cross-linker ratio than typical (1:40 instead of 1:60) was also evaluated. The imprinting factor of each was determined by comparing each imprinted polymer with a non-imprinted polymer prepared in a similar manner but with the absence of template.

In order to know the binding ability and to find out the optimum conditions for the template to be recognized by the MIP that being prepared, a standard solution of glibenclamide was initially prepared in various solvents such as chloroform, methanol, and

acetonitrile in a different pH condition. The filtrate that is indicated the amount of unbound analyte was measured. The results can be seen in Fig. 2.

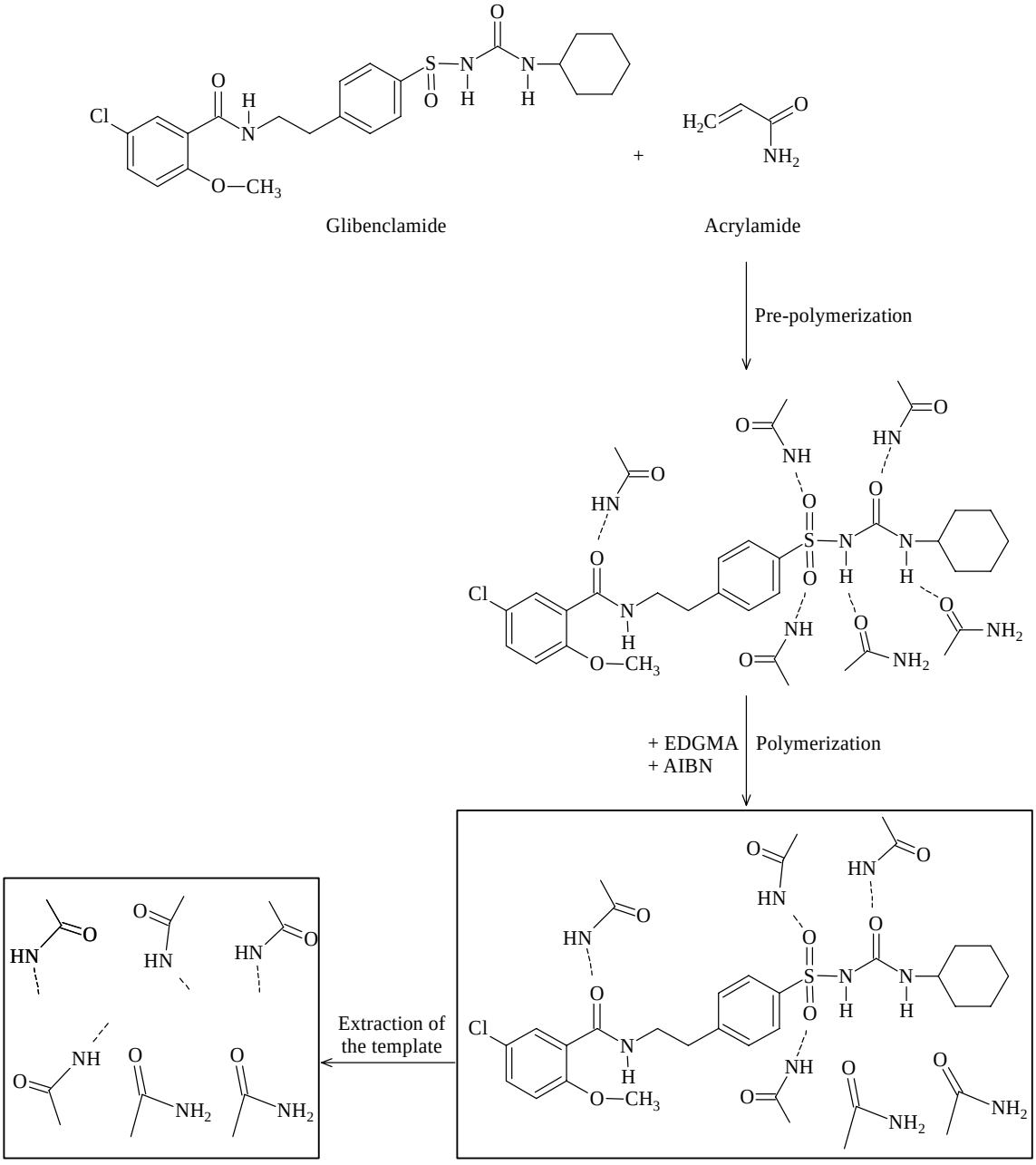

Fig. 1: Schematic Illustration of glibenclamide molecular imprinted procedure

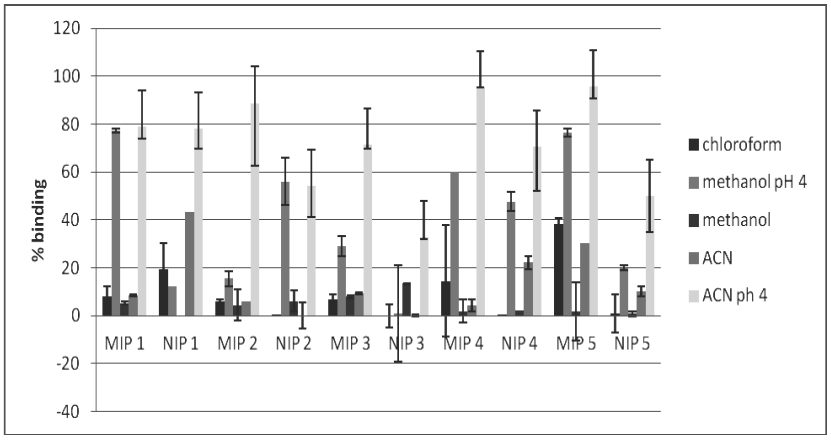

Fig. 2: The effect of acetonitrile, chloroform and methanol as the rebinding solution on glibenclamide rebinding percentage using MIP and NIP (mean ± SD, *n* = 3)

From Figure 2, it is known that MIP 4 can bind glibenclamide in 100% acetonitrile pH 4 but NIP 4 also produces a large percent of the binding that 76.23%. This suggests that the imprinting process is not very well distinguish analyte binding ability. The phenomena is may be due to low noncovalent interaction because of low monomer concentration. MIP 3 bind 88,81% of glibenclamide in acetonitrile pH 4 compare to 54.34% for NIP 3 one. This result showed that higher noncovalent interaction opportunity because of higher monomer concentration will lead to higher binding ability. This result is fit with the research done by Tom et al.[9]

Table 2: Imprinting factor (IF) of each polymer for glibenclamide

Imprinted polymer	IF (K_{MIP}/K_{NIP})
MIP 1	3.22
MIP 2	1.63
MIP 3	6.85
MIP 4	1.33
MIP 5	1.11

Of the five polymers, MIP 3 resulted in the highest IF value (6,85), this polymer had a higher ratio of monomer: template (6:1) with a cross-linker ratio of 70. These result may be due to glibenclamide molecule that is has at least 6 location of potential hydrogen bonding and adding sufficient acrylamide should maximize the interaction between

glibenclamide and acrylamide and improve the quality of the imprinted sites in the polymer. MIP 3 is also has highly cross-linked (70) so the polymer formed was rigid, and this is maximizing the binding-site integrity. MIP 5 with the highest monomer: template ratio (15:1) showed reduced IF value (1,11), this result because of excess monomer in this MIP increased the number of EDGMA-acrylamide or acrylamide-acrylamide reactions during polymerization. This mechanism would reduce the number of specific glibenclamide-acrylamide interactions, which did not allow sufficient glibenclamide site to form in the polymer, resulting in the limited ability of the polymer to rebind glibenclamide during use.

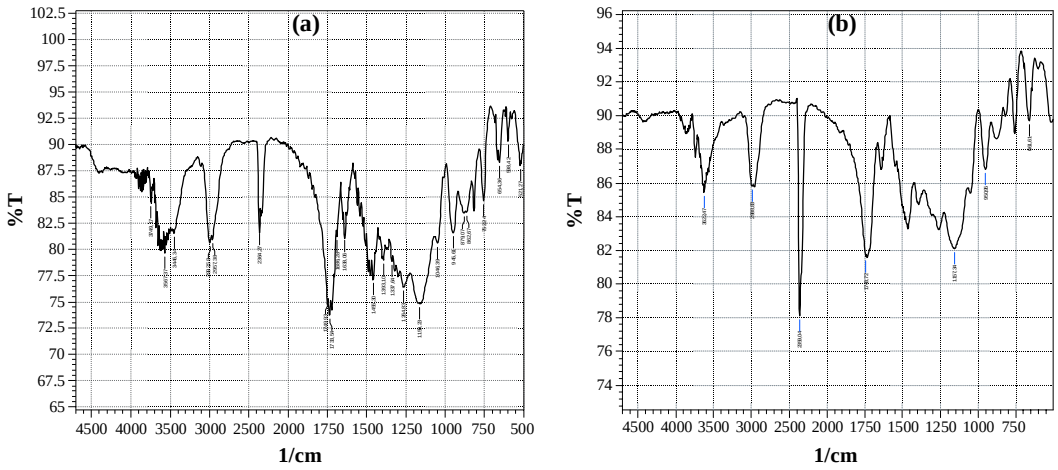

Fig. 3: FTIR Spectrum of MIP 3 (a) before and (b) after extraction of the template

From the results in Figure 3, we found a frequency shifting in C=O and NH of MIP 3. Before extraction the frequency is at 1733.54 cm^{-1} and 3567.37 and after extraction 1743.72 cm^{-1} and 3622.47, which indicates hydrogen bonding between the template and monomer. Before the extraction, because of the hydrogen bonding between template and acrylamide, there is a reduction in electron density in NH and C=O, which causes a reduction in vibrational frequency[14]. According to the results, MIP 3 has the potential for use in SPE for purification and concentration of glibenclamide and with further optimization.

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