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Research Article

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Synthesis of selective molecularly imprinted polymer for solid-phase extraction of glipizide by using a pseudo-template

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ABSTRACT

Selective molecularly imprinted polymers (MIPs) for solid-phase extraction and determination of glipizide have been designed and prepared. MIPs were synthesized with acrylamide as the functional monomer and ethyleneglycoldimethacrylate as the cross-linker in chloroform as a porogenic solvent by using bulk polymerization method. MIPs are able to bind 100% glipizide in chloroform by using a batch method compared to a non-imprinted one. By using Fourier Transform Infra Red, it was found that hydrogen bonding occurred between the carbonyl group of acrylamide and the proton donor of template. These results find the opportunity for this MIP to be used as an alternative of the pretreatment method before determination of glipizide by using glibenclamide as a pseudotemplate.

Keywords: molecularly imprinted polymer, pseudo-template, solid-phase extraction, sulfonylurea drugs, glipizide.

INTRODUCTION

Glipizide is a sulfonylurea class of antidiabetic drug that can lower blood glucose levels by stimulating the release of insulin from β-cells [1]. This compound is the most potent drug amongst the sulfonylureas [2]. Analytical techniques such as reversed-phase high-performance liquid chromatography [3,4] ion-pair high-performance liquid chromatography [5] and mass spectroscopy liquid chromatography [6] have been developed and used to monitor glipizide level in plasma. These methods have complicated procedures for pretreatment sample and high costs of instruments because of the sample matrices. All the methods need liquid-liquid extraction for the sample separation method. This kind of extraction has a problem in recovery of the sample, needs a lot of time, and has high solvent consumption [7,8].One of the separation methods that is popular is solid-phase extraction (SPE), which is faster, and more efficient than liquid-liquid extraction. However, SPE has low selectivity and this becomes a problem when it deals with complex biological samples or environmental samples or if analyte present in low concentration [8]. Higher selectivity of the sorbent can be increased by using molecular imprinting technique [9].

Molecular imprinting is a simple technique for preparing tailor-made affinity adsorbents that posses specific binding sites within polymer matrices. MIPs has an excellent properties to separate many interesting compounds [10]. MIPs are cross-linked synthetic polymers obtained by copolymerizing a monomer with a cross-linker in the presence of a template molecule (print molecule) in a solvent as a porogen. The polymer, with its template being washed away, contains recognition sites that are complementary in size, shape, and chemical functionality to the template molecules. Thats why the produced imprinted polymer is able to rebind selectively with analyte or its analogous structures. MIPs has a several advantages which is low cost, ease of preparation, and good physical and chemical stability over a wide range of experimental conditions and solvents like extreme pH. MIPs able to bind specifically to their original and related templates, and having tolerance to mechanical stress, temperature, pH, acid-base, etc. [11,12].In order to improve the properties of MIPs being developed, computer-aided study of MIP has

been used as a rational, fast and good method to search for optimal polymerization condition [13]. The characterization of molecular complexes formed between templates and monomers, with the aim of achieving a clearer picture of the interactions that are the basis of MIP technology, has been the goal of numerous theoretical studies [8,14,15].

In this study, we explore the utilization of MIPs as recognition units for glipizide by using acrylamide as a functional monomer in chloroform as a solvent. After that, we used a computational study to measure the energy of template-monomer interaction.

EXPERIMENTAL SECTION

Chemicals

Acrylamide (AAM), ethylene glycol dimethacrylate (EGDMA), 2,2-azoisobutyronitrile (AIBN), methanol, and chloroform were provided by Aldrich. Glybenclamide (GC) was provided by Hexpharm Pharmaceuticals Industry. Glipizide (GP) was purchased from Indonesia National Agency of Drug and Food Control.

Preparation of the molecularly imprinted polymers

The MIPs (Molecular Imprinted Polymer) and NIPs (Non Molecular Imprinted Polymer) were prepared by bulk polymerization. Briefly, molecularly imprinted polymers were prepared as follows: glibenclamide as a template (0.25 mmol), acrylamide as functional monomer (1.5 mmol), cross-linker EGDMA (16 mmol), and initiator AIBN (0.082 mmol) were dissolved in chloroform (4.5 mL) in a glass tube. The homogenous solutions were purged with nitrogen for 5 min, and sonicated for 40 min. Then the mixtures were incubated in the waterbath at 60°C for 24 h. The final bulk rigid polymers were ground in a mortar with a pestle and wet-sieved into mesh 60. Finally, the particles were extracted to remove the glibenclamide as a template in Soxhlet apparatus (24 h, chloroform:methanol (2:1)), and dried at 60° C for 18 hours and stored at ambient temperature. Complete removal of the template was ensured by monitoring the amount of template remaining in the extraction solvent by UV spectrophotometry and Thin Layer Chromatography. The non-imprinted polymers were prepared in a similar manner as used for the imprinted polymers but without template molecule during polymerization.

Evaluation of polymers

The Binding Batch-mode Experiments

The binding batch-mode experiments were performed in methanol and chloroform. Binding analysis was carried out by incubating 20 mg of polymer in 5 mL volume of the glipizide solution (10 µg/mL) in a glass vial and oscillated by a shaker for 3 h at room temperature at 120 rpm. The mixtures then were filtrated and an aliquot of solvent was used to analyze the amount of glipizide, not bound to polymer by UV-VIS spectrophotometry. The amount of glipizide bound to the particles was calculated by subtracting the amount determined after the experiment from the starting amount of glipizide in standard solution.

Molecular Modeling Analysis

The molecular models of the template molecules, functional monomer, and their complexations were drawn using ChemDraw and Chem 3D Ultra 8.0.3 (Cambridgesoft Corporation, USA). The 3D structures were drawn and Cartesian coordinates of stable conformers were generated to prepare an input file for running Gaussian 09 (Gaussian Inc., Wallingford, CT) simulations. The Hartree-Fock level of theory in combination with the 6-31G(d) basis set was used for geometry optimization to obtain structures with minimum energy. The possible modes of interaction between template molecules and functional monomers were sampled by manually docking ACM to the template molecule 1: 1. The Gibbs free energy gains of the complexes were calculated using eq. 1:

$$\Delta G = G_{\text{template-monomer complex}} - | G_{\text{template}} + G_{\text{monomer}} |$$

Where ΔG is the change in Gibbs free energy on the formation of template-monomer complex, $G_{\text{template-monomer complex}}$ is the Gibbs free energy of template-monomer complex, G_{template} is the Gibbs free energy of template, and G_{monomer} is the Gibbs free energy of monomer molecules.

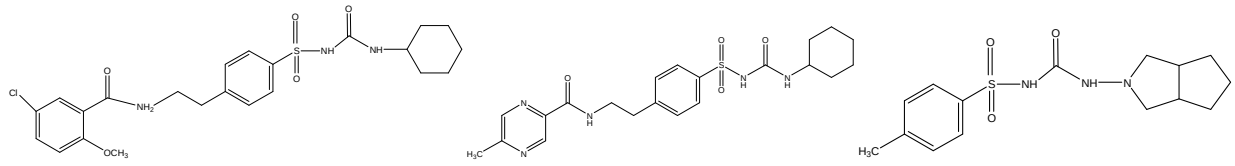
Characterization of Polymers

The functionality groups of the MIP and NIP and the presence of hydrogen bonding were determined by Fourier Transform Infra Red (FTIR).

RESULTS AND DISCUSSION

Preparation of the molecularly imprinted polymers

To do this study, first we had to decide the compounds to be used as template. The template is a compound that has a big role in this MIP to have molecular recognition properties. but, when the MIP is used for trace analysis, e.g., in plasma, template leakage will influence the accuracy of detection method. A pseudo or analog template, a compound which has the similar spatial structure with the target molecule, is an effective substitute for this these problems [15] Because of that, we use another compound from the sulfonylurea group that has similarity with glipizide as the target compound. According to the structure, glibenclamide has high similarity with glipizide. That is why glibenclamide then used as a pseudo template. The structure of the compound can be seen in Figure 1.



(a) **(b)** **(c)**

Figure 1. Structure of Glibenclamide (a), Glipizide (b), and Gliclazide (c)

The MIP with high affinity for glipizide was prepared by a bulk polymerization method in this work. In the non-covalent imprinting approach (in this case), hydrogen bonds play an important role in the recognition process [17]. From the study, We assumed that hydrogen-bond interactions between carbonyl group of the functional monomer and –NH group of the template were responsible for the selective binding of glipizide. Before and during polymerization, the non-covalent pre-polymer complex is affected by the polarity of the solvent. Less polar solvents, such as chloroform, will increase the complex formation, because it can facilitating a polar non-covalent interactions such as hydrogen bonding. Less polar solvents will eliminate other non-specific hydrophobic interactions and create a better environment for hydrogen bonding [12,18].Because of that,in this work, chloroform were used as the polymerization solvents for optimization of the molecular imprinting procedure. After the polymer is obtained, we do the extraction process of the template by using the Soxhlet method.

Evaluation of polymers

Stationary binding experiments

One of the best methods for evaluating the binding sites in MIPs is stationary binding experiment by using the batch adsorption test. Batch adsorption involves analysis of an MIP in a solution of substrate. Binding analysis was carried out by incubating 20 mg of polymer in 5 mL volume of the glipizide solution in different solvent (10 µg/mL) in a glass vial and oscillating in a shaker for 3 h at room temperature. We used chloroform, methanol, methanol pH 4 (methanol with acetic acid added until pH 4), and methanol pH 8 (methanol with ammonia added until pH 8).

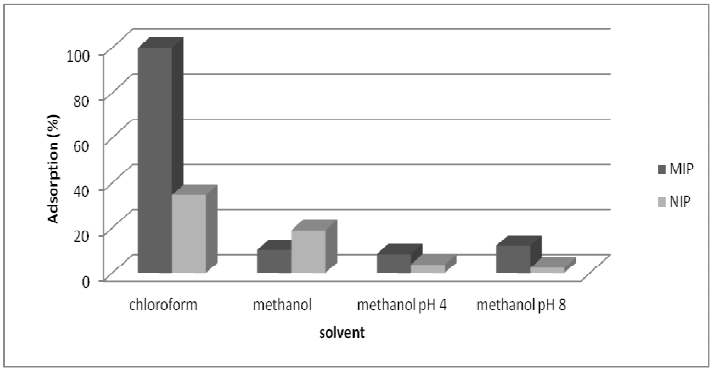


Figure 2. Binding Analysis Result of Glipizide

From figure 2, we found that the best adsorption is achieved by using chloroform as the adsorption solvent. This result is similar with Tamayo et al. [18], who showed that MIP has better recognition properties when the solvent is the same with MIP preparation.

Molecular Modeling Analysis

In recent years, the computational methods for designing molecularly imprinted polymers have been widely used. The studies to date have used these methods to describe the interactions present in the pre-polymerization mixture, in particular is an assumed interaction between the template molecule and the functional monomer [8] After we synthesized the MIP and did the adsorption test, we did the computational design for MIP. In this work, we computationally designed a MIP for glipizide by using Hartree Fock with a 6-31G(d) basis set. Conformation optimization was performed by Hartree-Fock with a 6-31G (d) basis set. According to the computational study, we found that ΔG value of the acrylamide-glibenclamide was 0.38 Kcal/mol. Glibenclamide is a pseudotemplate that we used for MIP preparation.

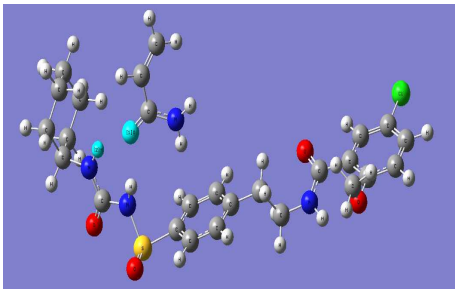
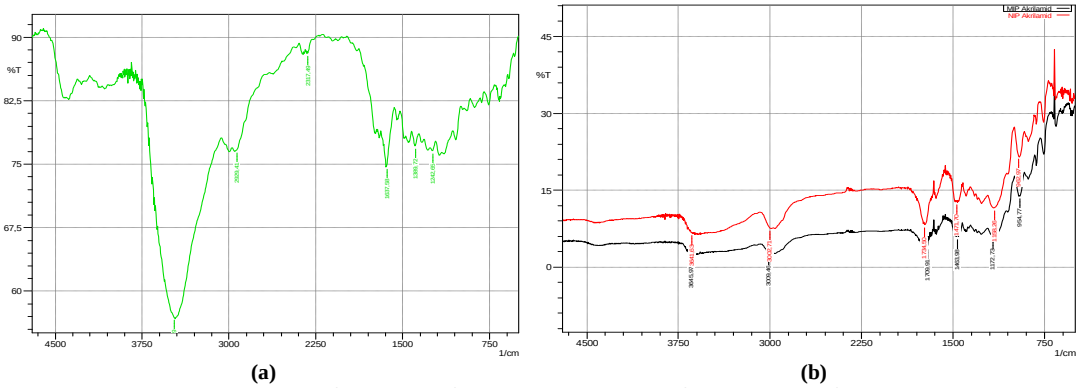


Figure 3. Visualization Result Interaction of AAM-Glibenclamide by using Gauss View

From figure 3, we found that glibenclamide has hydrogen-bond interactions with the carbonyl groups of AAM with 2.21581 angstrom**.**

Characterization of the Polymer

To find out whether there is hydrogen bonding present in the MIP, we used FTIR analysis. From the results in Figure 5, we found frequency shifting in NH and C=O: before extraction the frequency is at 3474.79 and 1637.58 cm^{-1}, and after extraction 3645.97 and 1709.91 cm^{-1}, which indicates hydrogen bonding between the template and monomer. Before 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 [20]. The frequency of MIP that is found by using FTIR is the same with NIP.



(a) **(b)**

Figure 3. FTIR Spectrum of Polymer Before Extraction (a) and After Extraction of the Template (b)

CONCLUSION

According to the results, there is a chance to use MIPs developed from acrylamide as functional monomers as alternative to the pre-treatment method for determination of glipizide.

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