Synthesis and characterization of meropenem imprinted polymer

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Abstract. Meropenem imprinted polymer (M-MIP) has been successfully synthesized. It is prepared by using meropenem (MERP) as template and acrylamide as functional monomer. For comparison, non-imprinted polymer (NM-MIP) was synthesized in the absence of MERP. The synthesized polymers were then characterized by infrared spectroscopy (IR) and thermogravimetry analysis (TGA). Extraction of template from polymer was conducted using Soxhletation method and methanol-acetate acid (85:15) as the leaching solvent. The prepared M-MIP showed higher adsorption capacity than the NM-MIP. Adsorption capacity of MIP and NIP were 4.8942 mg/g and 1.0078 mg/g respectively.

1. Introduction

Meropenem, (4R,5S,6S)-3-[(3S,5S)-5-dimethylcarbamoyl pyrrolidin-3-yl]-thio]-6-[(1R)hydroxyethyl]-4-methyl-7-oxo-1 azabicyclo[3,2,0] hept-2-ene-2-carboxylic acid, is a new parenteral carbapenem antibiotic with a very broad spectrum of antibacterial activity against the majority of gram-positive and gram-negative pathogens\cite{1}. Meropenem showed excellent efficacy in clinical studies involving seriously ill patients with intraabdominal, central nervous system, lower respiratory tract, skin and soft tissue, urinarytract, and febrile neutropenic infections\cite{2}. Meropenem demonstrated to be unstable in aqueous solution when submitted to thermal and alkaline treatment. Because of the instability of meropenem, special care must be taken to avoid exposure of the drug to the degradation conditions during the handling and storage of the pharmaceutical preparation \cite{3}.

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{meropenem.png}
\caption{Chemical structure of meropenem}
\end{figure}

In this study, we intend to synthesis sorbent for solid phase extraction of meropenem by molecularly imprinted polymer (MIP). MIP is a technique to design artificial receptors with a predetermined selectivity and specificity for a given analyte, which can be used as ideal materials in various application fields. Molecularly Imprinted Polymer (MIP), the polymeric matrices obtained using the imprinting technology, are robust molecular recognition elements able to mimic natural
recognition entities, such as antibodies and biological receptors, useful to separate and analyze complicated samples such as biological fluids and environmental samples. MIP synthesized by a non-covalent imprinting approach, which exhibit more selective recognition sites and higher adsorption capabilities for specific analytes or groups of structurally related species in contrast to the conventional sorbents. Molecular imprinting is a process where the target molecule acts as a template around which interacting and cross-linking monomers are arranged and copolymerized to form a cast-like shell (Fig. 2). Initially, the monomers form a complex with the template through covalent or non-covalent interactions. After polymerization and removal of the template, binding sites are exposed that are complementary to the template in size, shape, and position of the functional groups, which are held in place by the cross-linked structure. In essence, a molecular memory is imprinted in the polymer, which is now capable of rebinding or adsorbing the template selectively [4].

Figure 2. General principle of molecular imprinting. A molecular template (T) is mixed with functional monomers (M) and a cross-linker (CL) resulting in the formation of a self-assembled complex (1). The polymerization of the resulting system produces a rigid structure bearing imprinted sites (2). Finally removal of the template liberates cavities that can specifically recognize and bind the target molecule (3).

2. Material and Method

2.1. Material and instrumentation

Meropenem powder for injection was obtained commercially and claimed to contain 500 mg and 1000 mg as anhydrous base. Meropenem reference standard, acrylamide was bought from Sigma Aldrich. Acetonitrile for chromatography, potassium dihydrogenphosphate p.a. and ortho-phosphoric acid analytical grade were obtained from Merck. Dimethylsulfoxide (DMSO), ethyleneglycol methacrylate (EGDMA) were also obtained from Merck. Ultrapure water pyrogen free (water for injection) was obtained from PT Ikafarmindo and was used to prepare all solutions for HPLC. All solutions were prepared daily. Infrared absorption spectra for polymer was obtained using Fourier Transform Infrared (FT-IR) Prestige21 (Shimadzu), evaluation of leached polymer is observed by HPLC (Agilen).

2.2. Synthesis of MIP

To prepare the M–MIP, 1 mmol of MERP and the appropriate amount of functional monomer (Table-1) were dissolved in 20 ml of DMSO. To this mixture then cross-linker (EGDMA) and initiator benzoyl peroxide (BPO) were added. There were sonicated for 5 min. The solution was degassed with a stream of pure nitrogen for about 10 min. The vessel was caped under nitrogen flow and transferred to oven. After that, it was heated at temperature maximum of 60°C during 4 h. The polymeric particles were dried at the temperature of 60°C and rinsed with acetone. A Non-imprinted polymer (NM-MIP) as control polymer was also prepared using an identical procedure without adding MERP. Table 1 shows the recipes of the polymers synthesized in this study.

2.3. Extraction of MERP from polymer

The template was removed by a soxhlet extractor using (methanol: acetic acid = 85:15) for 24 h. The leached polymer particles were sieved to obtain particles sizes between 60 and 80 mesh.

2.4. Characterization of MIP
Characterization was conducted using Infrared Spectrophotometer for functional group analysis and TGA for thermal analysis.

2.5. Imprinting Factor (IF) calculation
M-MIP and NM-MIP were selected as competitive compounds to estimate the selectivity of M-MIP to meropenem. The analyte were dissolved with ultrapure water in 25 ml clinical flask. After adding 20 mg of M-MIP or NM-MIP, the suspensions were incubated at room temperature with shaking for 24 hour, and then the supernatant was analyzed by HPLC. The imprinting factor (IF) was expressed by the following formula:

\[
IF = \frac{Q_{M-MIP}}{Q_{NM-MIP}} \quad (Du, 2014)
\]

Where \( Q \) represent the adsorption amount of M-MIP or NM-MIP
Where \( Qe = \frac{V (C_0 - Ce)}{m} \) (Du, 2014)

3. Result and Discussion

3.1. Synthesis of M-MIP and NM-MIP
The composition of M-MIP and NM-MIP was as follows:

| Table 1. Composition of the polymer [5] |
|---------------------------------------|
| Template     | MERP     | 218.5 mg |
| Crosslinker  | EGDMA    | 3.75 ml  |
| Solvent      | DMSO     | 20 ml    |
| Initiator    | BPO      | 100 mg   |
| Functional Monomer | Acrylamide | 355.4 mg |

As we can see, NM-MIP was different from M-MIP. NM-MIP was white and jelly-like, M-MIP was yellow and jelly-like. The different between NM-MIP and M-MIP physically seen from the color. NM-MIP was white; M-MIP was yellow.

![Figure 3. Polymer (a) NM-MIP and (b) M-MIP (4) These polymers were then dried for several days in the temperature of 60°C after they were rinsed with acetone several times to pull the solvent that still left in the polymers out.](image)

3.2. Characterization of M-MIP
The infrared spectra of M-MIP showed in fig. 4. The important absorption bands observed was at 1152 cm\(^{-1}\) which correspond to C-N stretching bond in \(\beta\) lactam, and also 1724 cm\(^{-1}\) which corresponds to the stretching of C=O bond in \(\beta\) lactam [6]
**Figure 4.** Infrared spectra of M-MIP

**Figure 5.** (a) meropenem in Methanol-acetic acid spectra in various concentration; (b). spectra of leached polymer
3.3. Extraction of template from polymer

After polymerization completed, extraction of the template would be the next step to make M-MIP. Suitable solvent must be found to leach the meropenem (template) well. The complete leaching process was observed using UV spectrophotometer. As shown in fig 5 (a), the peak of meropenem was found at \( \lambda = 309 \text{ nm} \). As we can see, the peaks was in different level of absorbance according to the different concentration of meropenem in the proper solvent of extraction (methanol-acetic acid) = (85:15). From the red and blue line, which is the spectra of leached polymer, in Fig. 5 (b) we can see that was no peak found at the \( \lambda = 309 \text{ nm} \). It indicated that no meropenem found. As the control, there is peak of black line in fig. 4b that gave certain absorbance from meropenem standard added.

3.4. Thermal analysis

In order to study thermal stabilities of M-MIP NM-MIP and after-leached M-MIP, the thermo-gravimetric analysis (TGA) was employed. Figure 6 below showed the resulting thermogram.

![TGA curve for (A) NM-MIP, (B) after leached M-MIP; (C) M-MIP](image)

The leached polymer showed only 10% weight loss at the temperature around 100-200˚C. It showed the loss of porogenic solvent involved in the synthesis process, DMSO. There was different treatment in leaching the polymer. Polymer was leached in the presence of methanol and acetic acid, the volatile solvent. DMSO evaporated quickly together with methanol-acetic acid. As the information, the melting point of meropenem is at 150-153 ˚C, as seen in line blue (C) and line black (A), on that area, there were different percentage of weight loss. Blue line had more weight loss compared to the black line. It might be the loss of meropenem (blue line).

3.5. Imprinting factor calculation

| Table 2. adsorption amount Between M-MIP and NM-MIP |
|-----------------|-----------------|
| \( Q_{M\text{-MIP}} \) | 4.8942          |
| \( Q_{NM\text{-MIP}} \) | 1.0078          |
| IF               | 4.8563          |

The selectivity for meropenem was 4.8563. These could be ascribed to the hydrogen-bond interaction between the meropenem and functional monomers in the specific recognition sites of the imprinted polymers. These results demonstrated that the selective recognition was built on the complementarities of functional groups, size, and shape between the analytes and recognition sites[7].

4. Conclusion

Molecularly imprinted polymer for meropenem has been successfully synthesized and was characterized by its IR spectrum, TGA curve and the imprinting factor calculation.
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