

## Selective Solid-Phase Extraction of Meropenem from Human Blood Plasma Using a Molecularly Imprinted Polymer

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**Abstract:** This study employed a selective and high adsorption performance for meropenem. Molecularly imprinted polymer for meropenem (MeIP) as the selective sorbent was prepared through a bulk polymerization reaction. Methacrylic acid, ethylene glycol dimethacrylate, benzoyl peroxide, and dimethyl sulfoxide were applied as functional monomer, crosslinker agent, initiator, and solvent, respectively. Scanning electron microscopy, thermogravimetric analysis, Brunauer-Emmett-Teller analysis, and Fourier transform infrared spectroscopy were used to characterize the morphology, pore size, and structure of imprinted polymers. The maximum adsorption capacity was achieved at pH = 3, after 4 h contacted, under 150 rpm, and 25 mg of polymer applied. The maximum adsorption capacity of MeIP for meropenem was 51.963 mg/L; the synthesized polymer had superior selectivity to meropenem compared to that of the other antibiotics (imprinting factor, IF = 2.58). Furthermore, the thermodynamic and kinetic analyses indicated that the results were in accord with the Freundlich model and the pseudo-second-order kinetic model, respectively. MeIP was selective in batch adsorption, and molecularly imprinted solid-phase extraction protocols were selective to meropenem. It was then applied to analyze meropenem in human blood plasma and resulted in  $78.52 \pm 2.71$  of recovery.

**Keywords:** meropenem; molecularly imprinted polymer; selective; solid-phase extraction

### ■ INTRODUCTION

Meropenem, chemically (4R,5S,6S)-3-[(3S,5S)-5-(dimethylcarbamoyl)pyrrolidin-3-yl]sulfanyl-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, is a broad-spectrum antibacterial agent from the carbapenem family, with a  $\beta$ -lactam ring on its main structure [1]. Meropenem is commonly used in a therapy or a treatment of infection or disease in both adults and children that is caused by single or multiple susceptible bacteria [2]. For intravena protocols, meropenem was found in human blood plasma at 23  $\mu\text{g/mL}$  and 49  $\mu\text{g/mL}$  after 30 min of injection for the dose of 500 mg and 1,000 mg respectively [3]. Currently, the misuse of antibiotics is becoming a great concern along with drug resistance arising in the target of a treated microorganism. There are several reports regarding the

build-up of resistance to antibiotics by microorganisms, highlighting a decline of antibiotic efficiency unless a more rational and controlled use of such compounds is established [4]. Some methods mostly based on UV and MS detection have been developed to quantify antibiotics in different matrices. The determination of antibiotics in liquid samples has been reported, which commonly used the solid-phase extraction (SPE) method [5-7]. SPE was favorable recently because of its simplicity, short time procedure and minimal of solvent consuming. The development of sample pretreatment of antibiotics, especially meropenem, using, for example, SPE and column switching methods have been reported to be due to the combination of SPE-chromatography for the determination of some kinds of medicine [8]. However, the extraction of antibiotics as analytes using the SPE method is rather difficult due to the lack of

selectivity. Recently, the use of a molecularly imprinted polymer (MIP), synthesized using the molecular imprinting method, as a sorbent has been evolved to enhance the high selectivity of SPE (MI-SPE). The MIPs use the functionality of a target molecule (template) by forming specific interactions with a matrix during polymerization to assemble their own recognition site. The mechanism involves the formation of interaction or bonds by the template molecule, and the functional element before locking-in or bonds then drives the formation of a matrix with a selective recognition site for the template [9]. Because of its specific recognition and high selectivity, MIP has been applied as a potential clean-up system in many different sample matrices. MI-SPE also exhibits other favorable properties such as low cost, high stability, reusability, and long-term storage [10-11]. Highly selective sorbents toward a large number of analytes of environmental and pharmaceutical interests can be prepared by molecular imprinting.

This study presented the preparation of meropenem imprinted polymer (MeIP), through bulk polymerization, using methacrylic acid (MA) as a functional monomer, ethylene glycol dimethacrylate (EGDMA) as a crosslinker, benzoyl peroxide (BPO) as an initiator, dimethyl sulfoxide (DMSO) as a porogenic solvent and meropenem (Merp) as a template molecule. The aim of this work was to find the selective adsorbent of SPE for meropenem identification in human blood plasma. The morphology and structure of MeIP were characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis; pores and the surface were analyzed using Brunauer-Emmett-Teller (BET). The adsorption characteristics of MeIP were also investigated. Then, it was coupled with high-performance liquid chromatography (HPLC); MeIP was employed for SPE and applied to determine the amount of meropenem in spiked human blood plasma. A polymer synthesis from MA, EGDMA, and DMSO as solvents had not been reported previously.

## ■ EXPERIMENTAL SECTION

### Materials

The chemicals used in this study were meropenem

trihydrate purchased from Bernofarm Laboratories Company, MA, acrylamide (Sigma Aldrich), EGDMA, DMSO and BPO. Acetic acid (Merck Chemicals), nitrogen gas, methanol, acetonitrile (Fulltime Chemicals), curcumin, cefadroxil, amoxicillin and penicillin G (Kalbe Farma) were also used. While the distilled water was purchased based on HPLC grade.

### Instrumentation

The Thelco Laboratory model 130D heater was used for polymer synthesis; FTIR (Shimadzu Prestige 21) was used to investigate the functional groups of polymers; UV-visible spectrophotometer (Agilent 8453 G1103A); scanning electronic microscope (Hitachi SU 3500); HPLC (Infinity Agilent Tech. 1260 series), was set at 298 and 309 nm; Restek column for chromatography (C-18 150x4,6); BET (NOVA-Quntrachrome Instruments 10.01) was used to analyze the surface area, pore size, and pore diameter; SPE procedure was carried out using Supelco Visiprep™ 24; thermogravimetric analysis was performed using NETZSCH STA 449 F1; shaker and pH meter.

### Procedure

#### Preparation of MIP, NIP, and MeIP

For the preparation of MIP, 0.5 mmol meropenem, and 5 mmol MA were dissolved in 15 mL DMSO. They were contacted for approximately 15 min, in the pre-polymerization process at room temperature and without velocity on shaking procedure. Then, 20 mmol EGDMA was added to the solution, followed by water sonication, and N<sub>2</sub> gas was streamed into the mixture for approximately 10 min. A polymer solution was heated at 65 °C for 4 h. After the polymerization completed and produced MIP. MIP was then separated from excess of solvent, dried at 65–70 °C. The templates were extracted from the polymer by soxhlation where a mixture of methanol:acetic acid (87.5:12.5 v/v) was used as the solvent to construct MeIP. The extraction was controlled using a UV spectrophotometer at  $\lambda = 298\text{--}300$  nm and HPLC at  $\lambda = 298$  nm to ensure that the template molecules were totally extracted from the polymer. A non-imprinted polymer (NIP) was synthesized with the same composition and procedure of producing MIP in

the absence of the analyte (meropenem). Adsorption of meropenem was done under batch adsorption process and also for continuous protocols to extract meropenem from human blood plasma through SPE cartridge.

### Characterization of polymers

The functional group and structure in MIP, NIP and MeIP were observed using an FTIR spectrophotometer from 4,000 to 500  $\text{cm}^{-1}$ ; morphology of the polymers was also observed using SEM with 2,000 times of magnification for each polymer. Thermal analysis of polymers was carried out at a heating rate of 10  $^{\circ}\text{C}/\text{min}$  from room temperature to 800  $^{\circ}\text{C}$  under an air atmosphere. The specific pore diameter, surface area, and average pore diameter were measured by ANNOVA instruments with a bath temperature of 77.3 K and a nitrogen atmosphere.

### Adsorption test

The stock solution of 1,000 mg/L of meropenem was prepared fresh daily before the analysis with distilled water of HPLC grade. The effect of pH on the adsorption capacity of the polymer was investigated by mixing the polymer with the analyte in a 0.03 M phosphate buffer solution with a variation pH of 3 to 8 for 24 h; a 40 mL 50 mg/L meropenem solution was mixed with 30 mg of NIP and MeIP. The final concentration and capacity were then calculated using the Eq. (1) [12]:

$$Q = V(C_0 - C_t) / m \quad (1)$$

where  $Q$  = adsorption capacity (mg/g),  $C_0$  = analyte concentration before adsorption (mg/L),  $C_t$  = analyte concentration after adsorption (mg/L),  $m$  = mass of sorbent (mg), and  $V$  = solution volume (mL). Shaking rate was also observed by mixing a 30 mg polymer with a 30 mg/L analyte solution in conical flask of 100 mL for 20 h at various rates: 100, 150 and 200 rpm at room temperature with a 0.03 M phosphate buffer solution adjusted at pH = 3. The mass of polymer for adsorption was also observed by mixing a 20, 25, 30, 50, 75, 100 and 125 mg of polymer with a 50 mg/L analyte solution in a conical flask of 100 mL for 20 h with a 0.03M phosphate buffer solution at pH = 3. The effect of contact time (1–10 h) was investigated by mixing a 50 mg/L analyte solution, 40 mL, 25 mg of the polymer, then adsorption capacity was determined. Initial concentration was also

studied with the variation of concentration applied were: 10, 25, 50, 80, 100 and 125 mg/L. Adsorption capacity was then determined. The adsorption capacity was then examined using a UV spectrophotometer. Selectivity was represented by the imprinting factor (IF) and selectivity coefficient  $\alpha$ , defined as

$$\text{IF} = \text{QMIP} / \text{QNIP} \quad (2)$$

and

$$\alpha = \text{IF}_{\text{analyte}} / \text{IF}_{\text{analog molecule}} \quad (3)$$

where QMIP is the adsorption capacity of MeIP, and QNIP is the adsorption capacity of NIP [12].

To investigate the adsorption kinetics, 25 mg of polymer, 25 mL-40 mg/L of meropenem solution were placed into a conical flask. The mixture was shaken at 25  $^{\circ}\text{C}$  at different time intervals (60, 120, 180, 240, 300, 360, 480, and 600 min), and the concentration of meropenem in the supernatant was then analyzed.

### Molecularly imprinted solid-phase extraction procedure

As much as 50 mg of MeIP particles were packed into a 1 mL syringe as the cartridge and capped with a fritted polyethylene disk at the bottom. The cartridge was consecutively preconditioned with 2 mL distilled water and 2 mL methanol, followed by loading of 1 mL of the sample solution. After that, the cartridge was washed with acetonitrile:water (95:5 v/v) and eluted with methanol:acetic acid (80:20 v/v). The desorption solvent (methanol:acetic acid = 80:20) gave a better percent adsorption value than (methanol:acetic acid = 90:10 v/v), but to maintain sorbent safety, an eluting solvent was chosen (methanol:acetic acid = 85:15 v/v). The flowrate was optimized with a variation of 0.25, 0.55, and 3.00 mL/min, then left for 5 min, and left again for 10 min. The concentration of the sample solution before and after its adsorption by the sorbent was analyzed using HPLC.

The optimization in SPE includes: the selection of conditioning solvents carried out on two types of solvents, namely methanol and methanol-water composition. Methanol provides a better percent recovery, so that methanol is chosen as the conditioning solvent. Furthermore, for the washing solvent the authors chose acetonitrile water (95:5 v/v) with the approach that meropenem dissolves well in water. By

selecting acetonitrile:water as the mobile phase, it is expected that meropenem will retain well in the sorbent, this is evidenced by the chromatogram produced after washing. For the elution stage, the same solvent as the desorbing solvent was chosen, which has been done previously.

### Sample preparation

A fresh drug-free human plasma sample was obtained from the Indonesian Red Cross Organization, (PMI) Bandung-Indonesia. It was frozen, thawed, and allowed to reach room temperature before usage. To reduce matrices, blood plasma was diluted in methanol and then centrifuged at 6,000 rpm at room temperature. Methanol fraction was taken for further use. As much as 1 mL of clean blood plasma spiked with 50 mg/L of meropenem in human plasma was allowed to pass the SPE column containing 50 mg of sorbent MeIP. Plasma was considered as the diluent to dilute the meropenem solution. The flow rate was adjusted to 0.25 mL/min.

The filtrate then placed in a clear bottle and analyzed using HPLC. The adsorption and recovery were calculated following these equations:

$$\% \text{ of adsorption} = (C_{\text{initial}} - C_{\text{after}}) / C_{\text{initial}} \times 100 \quad (4)$$

$$\% \text{ recovery} = C_{\text{found}} / (C_{\text{initial}} - C_{\text{after}}) \times 100 \quad (5)$$

$C_{\text{initial}}$  is the concentration of the prepared analyte solution before its adsorption by MeIP,  $C_{\text{after}}$  is the concentration after adsorption by the polymer through the SPE column, and  $C_{\text{found}}$  is the concentration found after adsorption of the analyte by MeIP that was extracted from the polymer using a solution of methanol:acetic acid (80:20 v/v).

## RESULTS AND DISCUSSION

### Synthesis of MIP, NIP, and MeIP

The synthesis of MIP was carried out through bulk polymerization. This method was chosen because it is very simple, easy-prepared and no need of special treatment. DMSO was chosen as the solvent because it is an aprotic solvent that serves as porogen. The analyte has good solubility in water and DMSO. Although it has a high boiling point, the polymer should be dried before used. A previous study reported that a suitable porogen for noncovalent polymerization must be aprotic. The

formation of interaction between monomer and the template are stabilized under hydrophobic environmental [13]. The MIP was yellow, and NIP was white because of no meropenem in its composition or backbone of the polymer. This work followed the noncovalent approach, where complete polymerization was followed by the process of crushing and sieving to small particle sizes from a macroporous polymer. The template was removed from the polymer by Soxhlet extraction with methanol:acetic acid (87.5:12.5 v/v) as the solvent for extraction. The extraction of meropenem was controlled periodically by measuring the amount of meropenem in the extraction solvent using a UV spectrophotometer until no analyte was found. The decrease of absorbance at 298–300 nm after time cycle of soxhlation means that the concentration of analyte (meropenem) was also decreased by the time. In Fig. 1, it can be seen that the specific peak of meropenem appeared at 300 nm (showed by the black line) meanwhile the red line shows that the peak was disappeared. Then it can be concluded that no more meropenem existed in the backbone of the polymer after the extraction. It means that meropenem has been successfully removed from the polymer, hence the polymer is ready to be used. The leached polymer particles were sieved to obtain particles with sizes between 60 and 80 mesh.

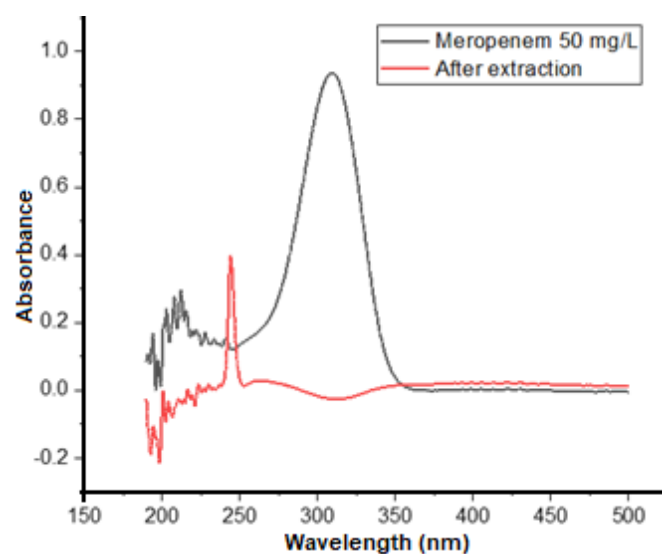


Fig 1. The spectrum of the analyte solution after analyte removal

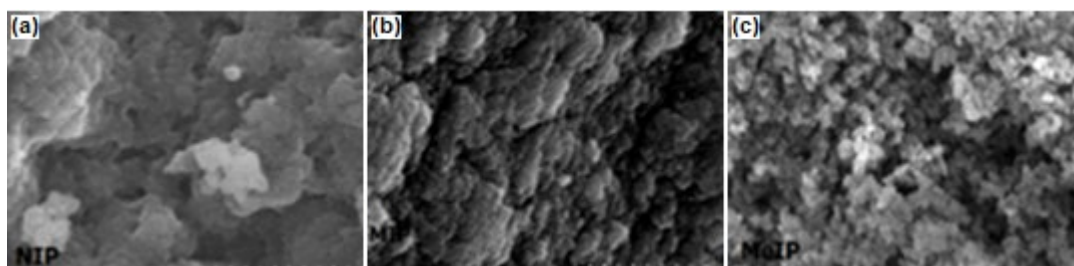


Fig 2. The morphology of (a) NIP, (b) MIP, and (c) MeIP characterized by SEM

**Table 1.** Adsorption capacity of NIP and MeIP

Sorbent	$C_0$ , ppm	$C_t$ , ppm	Q, mg/g	IF
MeIP	25	18.3235	5.3412	2.589
NIP	25	22.4216	2.0627	

To confirm the adsorption capacity of synthesized and extracted polymers, a batch-binding experiment was employed. As much as 30 mg of polymers (MeIP and NIP) were placed in a 100 mL conical flask and mixed with 30 mL of the analyte solution 25 mg/L which diluted with demineralized water under room temperature. Table 1 presents that MeIP had higher adsorption capacity than NIP. When the selectivity equals to 1, there is no adsorption capacity difference between MeIP and NIP. When the binding of MeIP > NIP, it indicates that MeIP has higher specific binding ability than NIP [14].

### Characterization of the Polymer

#### Morphology characterization

The surface morphology of NIP, MIP, and MeIP was characterized by SEM with 10,000 times of magnification (Fig. 2). As seen on the SEM image, remarkable differences in the morphologies of the polymers were considered, and a porous surface could be manifestly observed in MeIP, whereas the surface of MIP was bulky because of the presence of meropenem in the formation of the polymer. The more porous surface of MeIP supports the higher adsorption of the analyte, meropenem.

#### FTIR analysis

To confirm the functional group of the polymers, characterization using FTIR analysis was done. As seen in the spectrum Fig. 3, no peak was found around  $1,600\text{ cm}^{-1}$ , corresponding to the peak for the vinyl group. The possible explanation is that all of vinyl was involved in the polymerization. At  $3,400\text{--}3,450\text{ cm}^{-1}$ , a broad peak of OH

was found in the MIP spectrum, which is different in sharpness compared to NIP and MeIP corresponding to the presence of assembled-OH from a monomer and the template (meropenem) in the process before extraction. Since the OH peak was only from the monomer, it was found that the peak was not broad after the template extraction. The peaks in this region were similar for NIP and MeIP. The presence of meropenem in the spectrum of MIP was detected at the wavenumber of  $1,018\text{ cm}^{-1}$  and  $950\text{ cm}^{-1}$ , where these peaks come from  $\beta$ -lactam for C-H stretching and C-N stretching [15].

#### Thermal characterization

The thermal characterization was accomplished to examine the stability of the material [16-17], illustrated in Fig. 4. All polymers smoothly decomposed from 100 to 200 °C because the excess of solvent loss and the complex decomposition process started at 270 °C. As seen in the thermogram, MIP was decomposed at 340 °C,

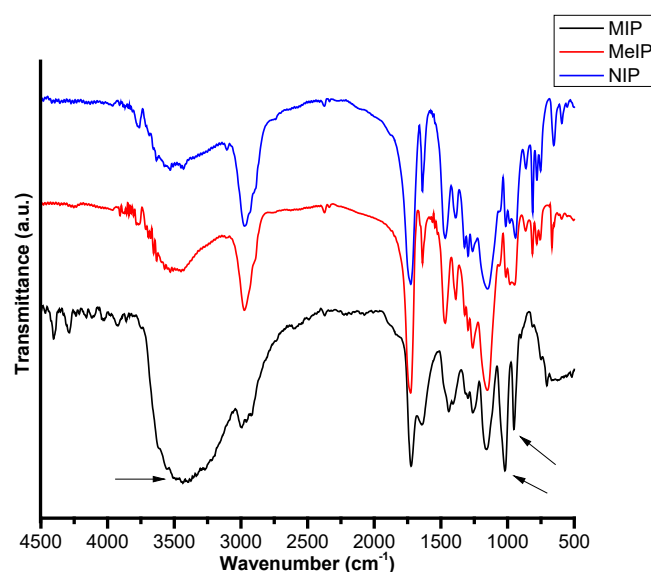


Fig 3. FTIR spectrum for NIP, MIP, and MeIP



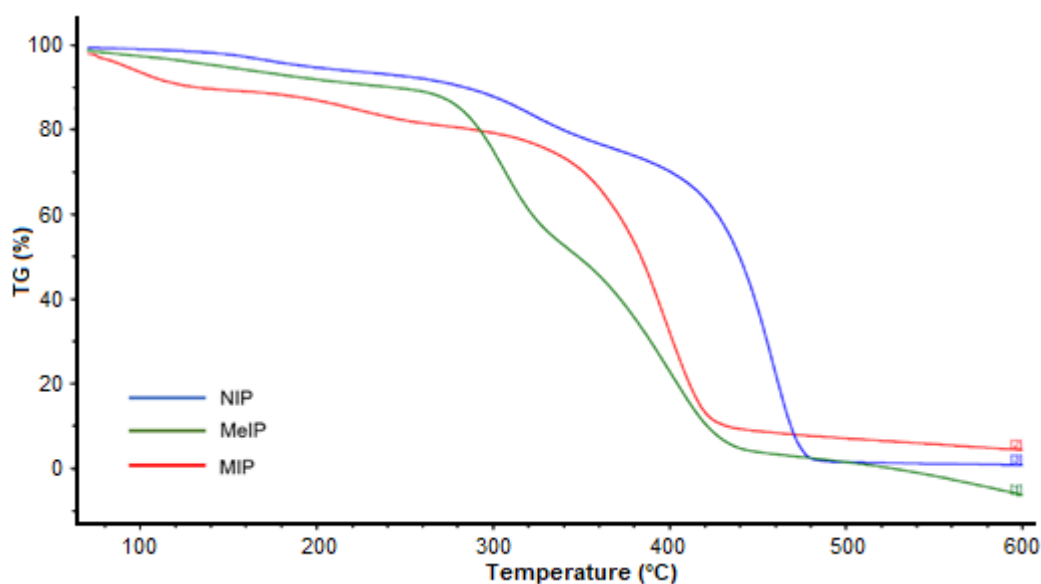


Fig 4. The thermogram of NIP, MIP, and MeIP

whereas the decomposition of NIP and MeIP started at a temperature under 340 °C. The possible reason for this phenomenon is that MIP had molecules of meropenem, which MeIP and NIP did not have, so the presence of meropenem leads to the necessity of higher temperature for its decomposition. Also, the difference in weight loss between MIP and NIP may be attributed to the temperature of meropenem degradation, which made the grafting density of the polymer different during polymerization.

#### BET analysis for specific surface area

Since specific surface area and pore size influenced the efficiency of MISPE adsorption, the parameters were obtained using a BET analysis. From Table 2, it can be seen that the pore diameter of MeIP was smaller, but the specific area and the pore volume were larger than those of NIP. Compared with NIP, MeIP has more pores. This phenomenon strongly indicated the effect of imprinting on polymerization. From the data, MeIP could provide more accessible cavities and binding sites for the target analyte than NIP. Therefore, the MeIP synthesized has benefited

Table 2. The results of BET analysis for NIP and MeIP

Polymer	Specific area, m <sup>2</sup> /g	Pore volume (cc/g)	Pore diameter (Å)
NIP	4.643	1.83 × 10 <sup>2</sup>	78.69
MeIP	47.08	6.43 × 10 <sup>2</sup>	27.27

from the conduction of interaction and has more accessibility to the adsorbate.

#### Adsorption Test

##### Effect of pH

Owing to the presence of OH<sup>-</sup>, H<sup>+</sup>, and NH in the structure of meropenem, as well as the pH value of meropenem (pKa1 = 2.9; pKa2 = 7.4) [18], the pH may influence its ionic form. That is why pH is important to be considered in studying the adsorption behavior of MA as the polymer has pKa of 5.4 [19]; thus, the high adsorption at pH 3 could be caused by the electrostatic interaction between meropenem in the anionic state and MA in the protonated state. The presence of a cavity also supported this high value. However, in pH 4, the adsorption was decreased because the positive charge on the MA surface was decreased. By contrast, when the meropenem met its isoelectric point of approximately 5.15 [20], it was attached to the sorbent surface by partition mechanism [21]. Further, the adsorption dropped with increasing pH because of the negative charges on both the sorbent surface and meropenem. Thus, the interaction was electrostatic repulsion instead. Predominantly, the adsorption of meropenem using MeIP resulted in a higher value than that using NIP because of by the cavity in MeIP. Fig. 5 indicated the decrease of adsorption (n = 3) because adsorption was

dependent on not only the charge of the analyte but also the charge of the surface of the functional monomer, MA [22].

### Effect of mass of the sorbent

The mass of the sorbent must affect the adsorption capacity because it represents the amount of the sorbent that can adsorb the analyte. Fig. 6 illustrates the dependence of the adsorption capacity on the mass of the sorbent ( $n = 3$ ). It can be stated that increasing the polymer mass increased the adsorption of meropenem. However, the adsorption remained constant when the mass of the sorbent was over 25 mg. This can be attributed to the saturation of the active site on the sorbent [23].

MeIP gave a higher adsorption capacity than that of NIP at the same mass considering the active site of the imprinting molecule. Meanwhile, the adsorption on NIP was caused only by the unspecific pore in the surface, contrary to the adsorption on MeIP, which is mainly caused by the active site. It can be concluded that the imprinting factor successfully gained.

### Effect of contact time

In the adsorption process, contact time is typically investigated in a different span of 1 to 10 h. In Fig. 7, 4 h-contact time gives the maximum adsorption of meropenem. The adsorption of meropenem onto MeIP always gave a higher value than that onto NIP because the active site on MeIP offered more adsorption than the

surface of NIP, which was only caused by the unspecific pore of NIP. However, MeIP met the constant state when the contact time was over 4 h. This indicated that the analyte had covered up the MeIP surface. Thus, the surface had been saturated and could not draw more analytes [24].

From Fig. 8, the kinetics model of meropenem adsorption onto MeIP fit to a pseudo-second-order mechanism that had a regression value of 0.8524. The adsorption involved electron exchange between the adsorbent (MeIP) and the analyte (meropenem). The

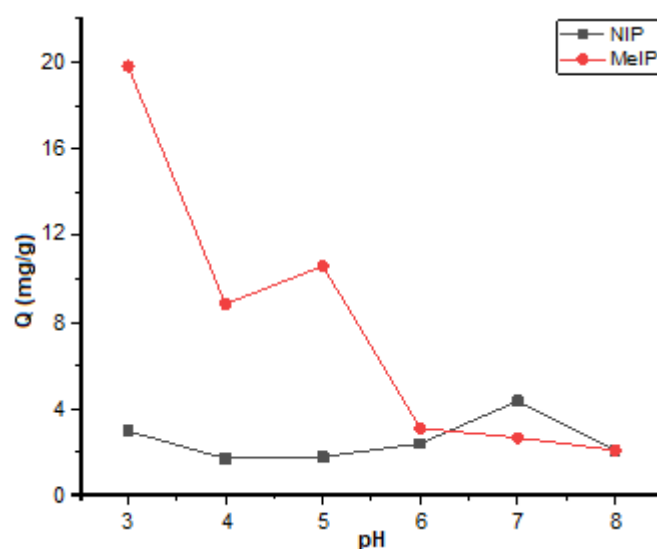


Fig 5. pH effect to adsorption capacity

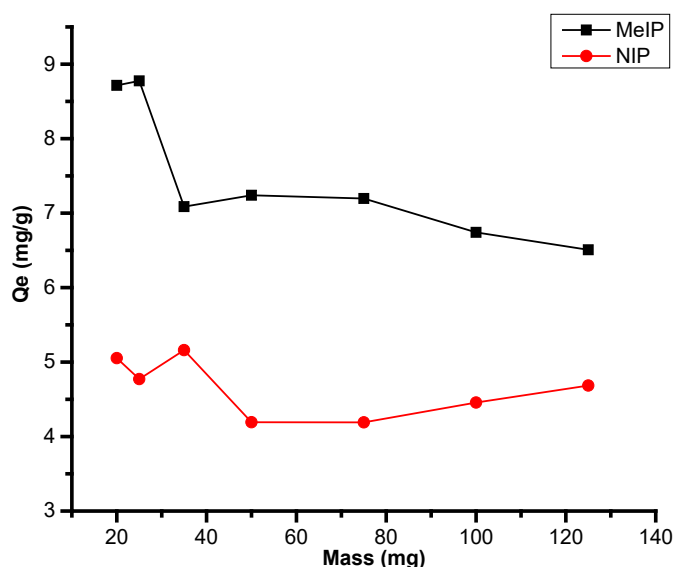


Fig 6. Effect of mass of the sorbent on adsorption capacity

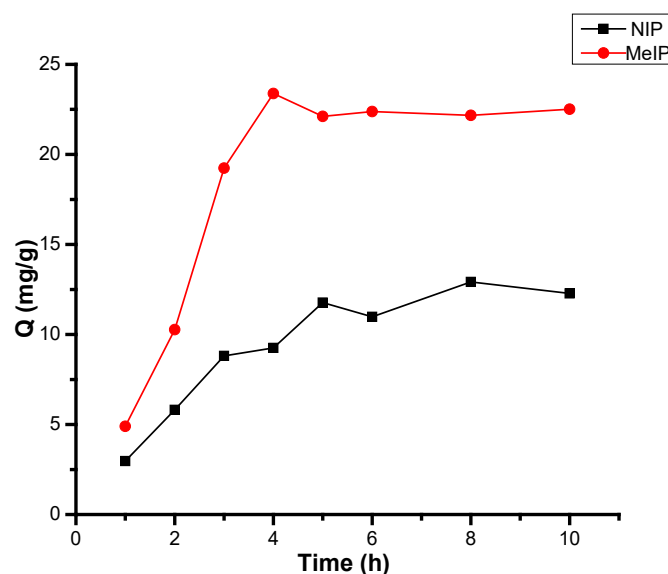
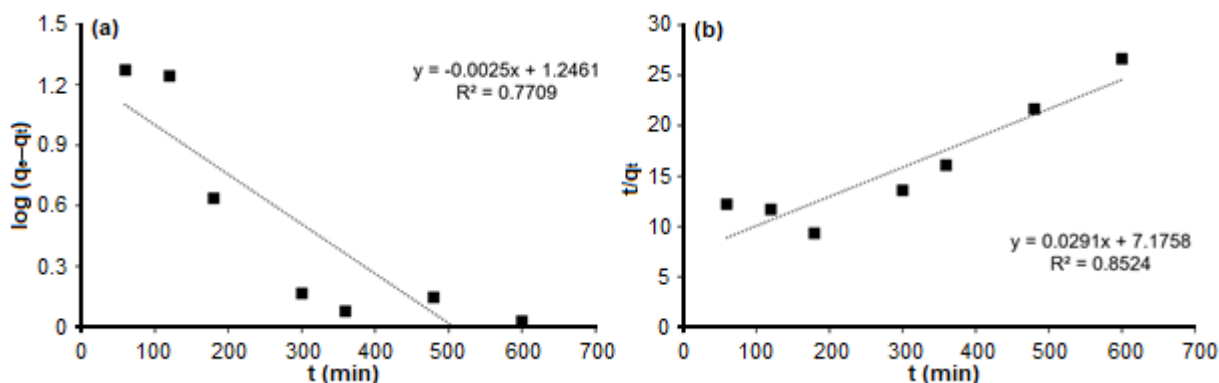


Fig 7. Effect of contact time



**Fig 8.** The (a) pseudo-first order and (b) pseudo-second-order kinetics model of the meropenem adsorption by using MeIP

pseudo-second order assumes that there is no desorption, so the back reaction is negligible. The concentration of the analyte involved in adsorption was considered to be stable because concentrations observed were those in the adsorbent, not in the analyte solution [25].

From Fig. 8, the kinetics model of meropenem adsorption onto MeIP fit to a pseudo-second-order mechanism that had a regression value of 0.8524. The adsorption involved electron exchange between the adsorbent (MeIP) and the analyte (meropenem). The pseudo-second order assumes that there is no desorption, so the back reaction is negligible. The concentration of the analyte involved in adsorption was considered to be stable because concentrations observed were those in the adsorbent, not in the analyte solution [25].

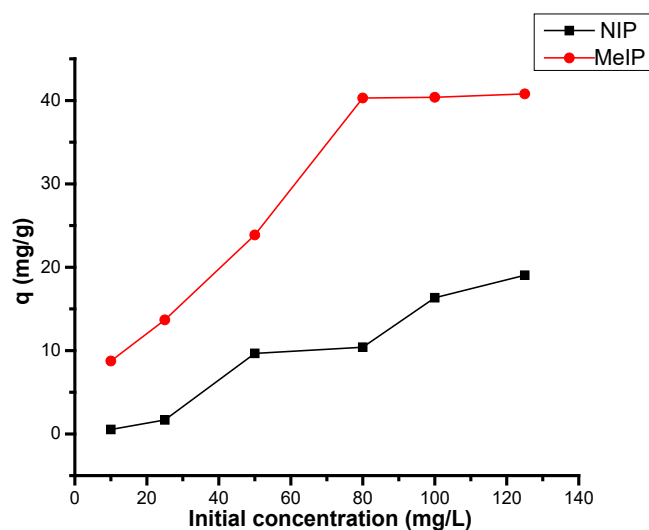
### Effect of initial concentration

As presented in Fig. 9, the adsorption capacity increased as the initial concentration increased until the concentration of 75 mg/L ( $n = 3$ ). The adsorption capacity remained stable, implying that equilibrium had been reached. MeIP capacity was higher than NIP capacity at any concentration, indicating that binding affinity of meropenem in the sorbent (MeIP) had been successfully obtained.

In this work, the adsorption isotherm was determined by the amount of the analyte after the adsorption process to determine the analyte adsorbed. Langmuir and Freundlich isotherm models were examined in this work. Langmuir assumes the monolayer adsorption; the active site in the surface of adsorbent has

homogen energy of adsorption at any area in the surface [26]. Meanwhile,  $K$ , in the isotherm model of Langmuir and Freundlich, depicts the amount of binding capacity between the analyte and the surface of the adsorbent (MeIP) [26]. The equilibrium data of MeIP were fitted to the Langmuir and Freundlich isotherm models. The correlation coefficient ( $R^2$ ) is used to study the applicability of the isotherm models to the adsorption behaviors.

The adsorption isotherm constants of MeIP are listed in Table 3. Furthermore, non-linear regression is used to compare the Langmuir isotherm model with the Freundlich isotherm model for adsorption of meropenem onto MeIP, and the results are illustrated in Fig. 10. The Langmuir isotherm model is used to describe monolayer

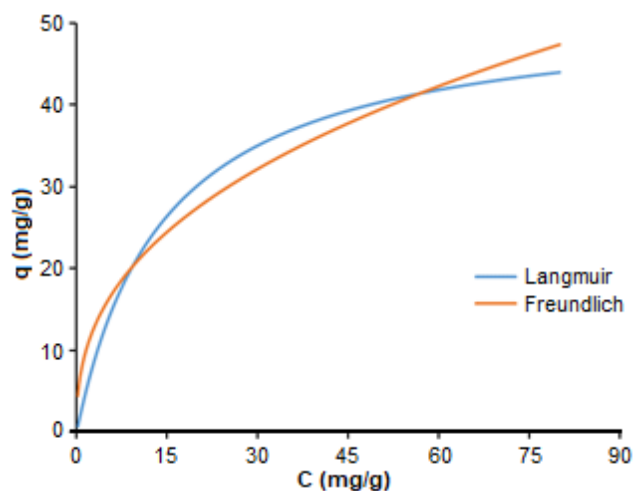


**Fig 9.** Effect of initial concentration



**Table 3.** Parameters of isotherms

$Q_{\max}$ mg/g	Langmuir Isotherm l/mg		Freundlich Isotherm	
	$K_L$	$R^2$	$K_F$	$R^2$
51.963	0.069	0.7963	8.437	0.9379

**Fig 10.** Isotherm model of the adsorption of meropenem using MeIP

adsorption on the adsorbent surface with limited identified sites [26]. The linear form is expressed by Eq. (6), and the Freundlich isotherm model is given as Eq. (7). An empirical equation is used to describe the adsorption process [27].

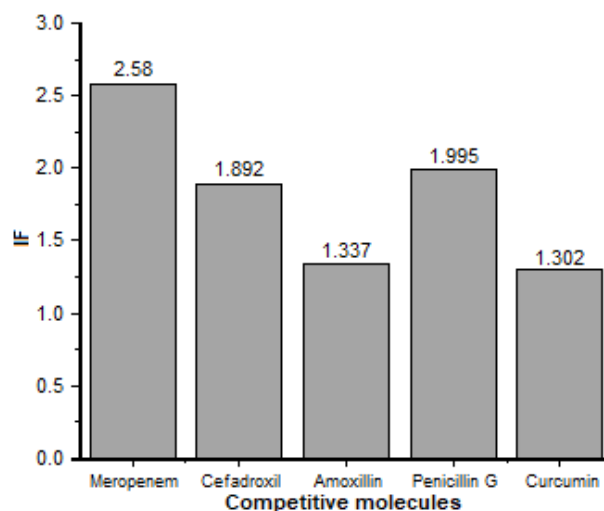
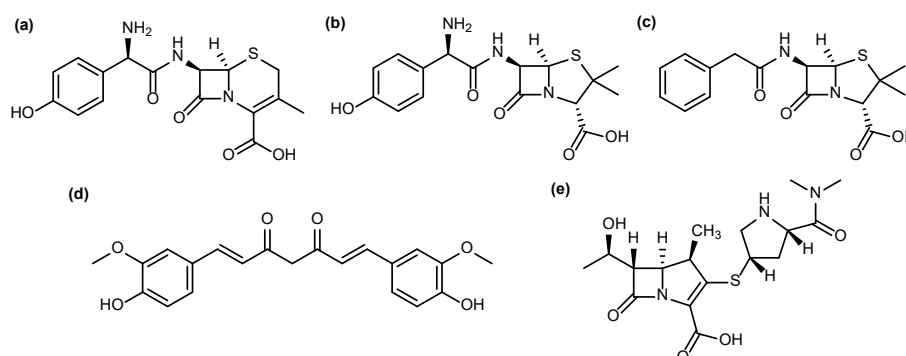
$$C_e / q_e = C_e / q_m + 1 / (q_m K_L) \quad (6)$$

$$\ln q_e = \ln C_e / n + \ln K_F \quad (7)$$

From Fig. 10, the data calculated was presented in Table 3. It can be confirmed that the adsorption of meropenem followed the Freundlich model.

### Selectivity test

The imprinting factor of meropenem was compared to those of other competitive compounds to determine the selectivity. The competitive compounds selected were cefadroxil, penicillin G, amoxicillin, and curcumin. Cefadroxil, penicillin G, and amoxicillin (see Fig. 11) were chosen because of the similarity of B-lactam in their main structure; curcumin was also investigated because its selectivity had to be examined as it was often prescribed together with antibiotics on therapy. The IF value is given in Fig. 12. The result indicated that the adsorption amounts of penicillin G and cefadroxil were high; this resulted from their structural similarity to meropenem. By contrast, MeIP had low affinity for amoxicillin; this might be because the optimum condition (pH and employed solvent) obtained for extraction of meropenem was not suitable for amoxicillin.

**Fig 12.** IF of meropenem and competitive molecule**Fig 11.** The chemical structure of competitive compounds: (a) cefadroxil, (b) amoxicillin, (c) penicillin G and (d) curcumin (e) meropenem

MeIP also had affinity to curcumin, a supplement compound that is given mostly with antibiotics; however, they have a different structure to that of meropenem. This might be caused by the unselective pores in the surface of the polymer, which accommodates the size of the molecule of curcumin. In the calculation of the imprinting factor, IF, the adsorption capacity of MeIP was higher than that of NIP, because of the absence of binding sites in NIP that also caused low adsorption and affinity.

### Thermodynamics of adsorption

The common thermodynamic parameters, i.e., the standard enthalpy change ( $\Delta H^\circ$ ), the standard free energy change ( $\Delta G^\circ$ ) and the standard entropy change ( $\Delta S^\circ$ ), were also determined in this study. Those were calculated using Eq. (8) and (9).

$$\ln K_c = -\Delta H^\circ / RT + \Delta S^\circ / R \quad (8)$$

$$\Delta G^\circ = RT \ln K_c \quad (9)$$

$K_c$  (mL/g) is a distribution constant and can be calculated by plotting  $\ln(q_e/C_e)$  versus  $q_e$  and extrapolating  $q_e$  to zero. The value of  $K_c$  is the intercept of the straight line;  $T$  is the absolute temperature (K), and  $R$  is the gas constant (8.3145 J/(mol K)). The obtained thermodynamic parameters for meropenem-MeIP for the adsorption process are listed in Table 4. A negative  $\Delta G^\circ$  value indicated that the adsorption of meropenem onto MeIP was spontaneous within the evaluated temperature range. A negative value of  $\Delta H^\circ$  confirmed that the adsorption process was exothermic. Therefore, decreasing the temperature can favor the adsorption of meropenem onto MeIP [28]. The more the meropenem molecules adsorbed onto the MeIP surface, the more solvent molecules surrounded meropenem molecules. Thus, the degree of freedom to meropenem molecules was decreased [29]. A negative value of  $\Delta S^\circ$  suggested that there was low-binding randomness at the solid-solution interface [28].

### MISPE Analysis

A 50 mg amount of imprinted polymer was packed into an empty SPE cartridge of a 1 mL analyte solution with frits at the bottom. The bleeding of the residual template from the polymer was checked by washing the MISPE cartridge with acetonitrile:water (95:5 v/v). The

chromatogram was free of meropenem. For the first step of extraction of meropenem, MISPE cartridges were conditioned with 3 mL of methanol and 3 mL of HPLC grade water, to wet the polymer completely before its usage; then, 1 mL of 40 mg/L of the meropenem solution was passed through at 0.25, 0.55, and 3 mL/min. After loading, the polymer was washed with acetonitrile. The target analyte was eluted from the polymer with 1 mL of methanol:acetic acid (80:20 v/v). The effect of the flow rate and treatment before elution applied on extraction is presented in Table 5.

The lower flow rate applied meant the longer time of the analyte in contact with the polymer that led to more the analyte being adsorbed. As seen from the table, although the polymer and the analyte were in the same cartridge but made no contact, it did not give an advantage for the adsorption.

Reusability study of the adsorbent is one of important parameter that can show an advantage of MIS-PE application. In this study, the investigation of MeIP reusability ( $n = 3$ ) gave the result as shown in Fig. 13.

Further, for the SPE study, we investigated the selectivity of the MeIP to amoxicillin. 50 mg/L for each meropenem-amoxicillin passed through the 1 mL SPE column with a flow rate of 0.25 mL/min. According to the literature, the modified optimum eluent was water:methanol:phosphoric acid: triethylamine (842:150:4:4 v/v/v), and the pH adjusted to 4 was the best

**Table 4.** Thermodynamic parameters of meropenem-MeIP

T (K)	$\Delta H^\circ$ (kJ/mol)	$\Delta G^\circ$ (kJ/mol)	$\Delta S^\circ$ (J/(mol K))
298		-26.229	
313	-71.465	-23.952	-151.797
328		-21.675	

**Table 5.** The effect of the flow rate applied ( $n = 3$ )

Flow rate (mL/min)	Adsorption (%)
0.25	82.59
0.55	77.01
3.00	28.66
Left for 5 min	26.11
Left for 10 min	26.02

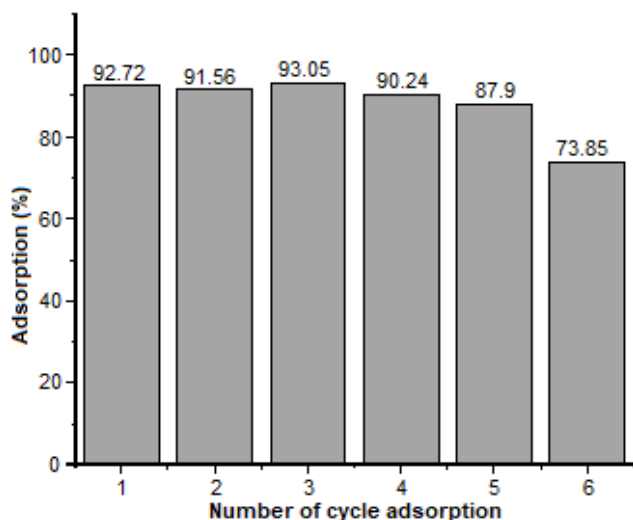


Fig 13. Reusability of MeIP for meropenem adsorption

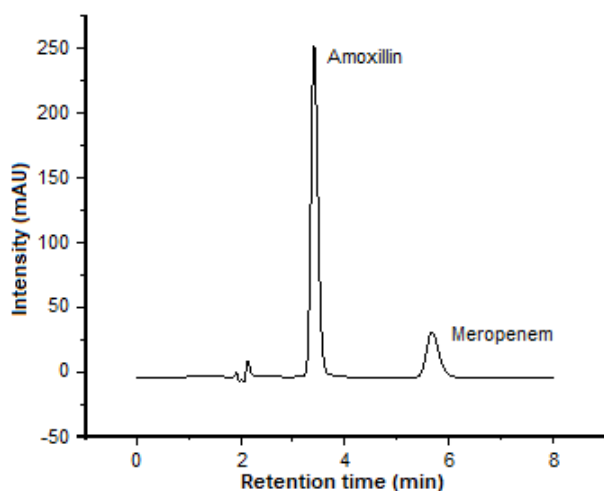


Fig 14. Chromatogram of meropenem-amoxicillin separation on SPE

Table 6. Summary of meropenem-amoxicillin adsorption on SPE (n = 3)

	meropenem	amoxicillin
$C_{\text{initial}}$ (mg/L)	50	50
$C_{\text{after}}$ (mg/L)	7.01	28.64
Adsorption (%)	85.98	42.72
$C_{\text{found}}$ (mg/L)	35.25	17.75
Recovery (%)	81.12	83.09

for the separation for meropenem-amoxicillin in HPLC [30]. The separation chromatogram and the recovery can be seen in Fig. 14 and Table 6, respectively; they indicate that the adsorption of MeIP to meropenem was two times higher than that to amoxicillin.

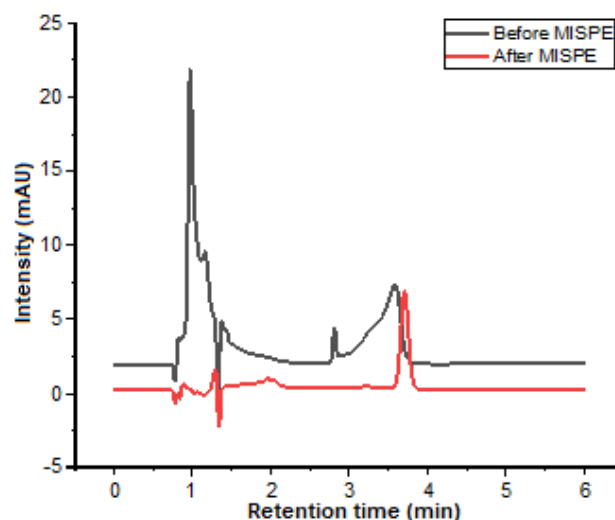


Fig 15. Chromatograms of meropenem before and after MISPE

Table 7. Determination of meropenem in plasma using MISPE (n = 3)

Spiked concentration	Adsorption (%)	Recovery (%)
40 mg/L	$76.85 \pm 6.18$	$78.52 \pm 2.71$

### Sample Preparation

The fresh human blood plasma was obtained from Red Cross Indonesia, Bandung region, Indonesia. Chromatograms of the analyte before and after MISPE application are presented in Fig. 15. The percentage of adsorption and recovery of meropenem are listed in Table 7. The percentage of adsorption seemed to be stable in three times of repetition, referred to the stability use (reusability) of the synthesized polymer.

### CONCLUSION

A novel molecular imprinted polymer (MeIP) for extraction and identification of meropenem using a polar solvent was successfully prepared. In this study, MeIP was prepared to quantify meropenem in human blood plasma. An MISPE procedure was applied by combining it with HPLC detection. This study has demonstrated good performance of adsorption capacity compared to NIP, high selectivity of competitive molecules, and good reusability of polymers to meropenem. These polymer particles can be promising materials for SPE and column packing materials.

## ■ AUTHOR CONTRIBUTIONS

LS conducted the experiment and analysis. MAZ, MB, AA examined the experimental and analytical data. LS wrote the original manuscript. All authors agreed to the final version of this manuscript.

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