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PREPARATION OF ANTIBACTERIAL BENTONITE β LACTAM ANTIBIOTIC COMPOSITE

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Abstract

The adsorption of Doripenem (beta-lactam antibiotic belonging to the subgroup carbapenem) from aqueous solution using bentonite as the adsorbent was studied at various pH of the solution. The doripenem loaded in bentonite which called delipenem-bentonite composite was subsequently used as the antibacterial agent. The effects of pH on the adsorption of doripenem onto bentonite was studied at pH 2 to 10 at an initial concentration of 200 mg/L and temperature of 30 27. The kinetics of adsorption was also studied, and the results were adjusted by pseudo-first-order and pseudo-second-order models, and the result indicates that pseudo-first-order gave better performance in representing the kinetics data of adsorption of doripenem onto bentonite. The adsorption isotherm of doripenem onto bentonite could be described well by the Langmuir model. Buffer solution at pH 3 gave better release of doripenem from the surface of bentonite. The microbiological testing results indicate that doripenem-bentonite composite has potential application to inhibit the growth of *Staphylococcus aureus* and other bacteria.

Key words: doripenem, adsorption, bentonite-doripenem composite

Introduction

An antibacterial is an agent that interferes with the growth and reproduction of bacteria. Inorganic antity terial using silver ions have long been known as an antibacterial which has strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities (Grier, 1983). However, silver has potentially toxic effects on human health and causes death in animals. As it has been recently reported in the literature, silver possibly has 50% more toxicity than chrysolite asbestos (Soto et al. 35 005). Organic antibacterial now much more popular than inorganic ones, and it is very effective to inhibit the growth and reproduction of bacteria. In modern society, unitibiotics are utilized to cure various kind of infections caused by bacteria, such as infected wounds, skin infections, urinary tract infections, among other applications. The purpose of this research is to

explore the potential application of bentonite as a drug carrier and to study the drug release in the solution. A number of studies have been conducted to combine antibiotic with other inorganic materials to form the antibiotic composites such as gentamycin-montmorillonite for bacterial inhibition (Rapacz et al., 2016), oxytetracycline-montmorillonite as excipients in pharmaceuticals formulations (Aristilde et al., 2016), montmorillonite-kaolinite-laponite-ciprofloxacin composite as medicine to deliver antibiotics across the skin (Hamilton et al., 2014), ofloxacinmontmorillonite-chitosan as control drug release (Hua et al., 2010), and tetracycline-montmorillonite as excipient in pharmaceutical formulations (Parolo et al., 2010). The antibiotic doripenem is the 16 vest carbapenem belongs to the betalactam group. Doripenem demonstrate the broadest spectrum of activ 16 among the β-lactam antimicrobials. Doripenem has the ability to against various gram-positive and gram-negative aerobic and anaerobic bacteria, including many multidrugresistant gram-negative pathogens. Improved potency against non-fermentative gram-negative bacteria has also been demonstrated by doripenem and in comparison with other carbapenems (Jones et al., 2005). Clay such as bentonite is the total material and has as its main component clay minerals (montmorillonite). The Bentonite composition is largely montmorillonite, which has been known as the good adsorbent. Montmorillonite is the most studied swelling clay mineral. It is a layered material with a negative structural charge due to isomorphic substitutions, which impart a cation exchange capacity (CEC) of about 100 mEq/1 g and hydrophilic properties to its surface (Bergaya and Lagaly, 2006). Also, this clay mineral presents colloidal particle size and high specific surface area. All these properties determine the adsorption of various inorganic and organic substances. These clay minerals provide materials to be used in the pharmaceutical formulation and as adsorbents of organic pollutants. Due to its excellence in adsorption properties, this clay mineral is widely used to adsorb various inorganic and organic compounds (Abollino et al., 2003). Some studies also employes bentonite-type clay minerals from La Pampa province 15 rgentina as drug carriers or drug excipients (Parolo et al 2008), three types of nanoclays (bentonite, octadecylamine-modified montmorillonite and halloysite) used as potential carriers for antimicrobial peptides nisin and pediocin (Meira et al., 2015), utilizing chitosan-montmorillonite hydrogel as release of loxacin (Hua et al., 2010) and other pharmaceutical applications. Although some studies have utilized clay minerals as one of the materials for composites preparation for pharmaceutical applications, however, there is no study on the utilization of bentonite-doripenem composite as an antibacterial application. This article aims to present a study of the adsorption of dorigenem on a Pacitan bentonite and subsequent application as an antibacterial agent to inhibit the growth

Materials and methods

of Staphylococcus aureus.

Materials

Doripenem with purity at 96% was obtained from PT. Dexa Medica, Palembang, South Sumatra, Indonesia and used without any further treatment. Bentonite was

acquired from Pacitan, East Java, Indonesia. Bentonite was purified using H_2O_2 to remove any organic impurities. The purification of bentonite was conducted at 30°C using 38% hydrogen peroxide solution. After the purification process completed, the suspension was heated at 110°C to remove the excess of hydrogen peroxide. The purified bentonite was dried in an oven for 24 h at 105°C. The purified bentonite was crushed into powder and ready to use.

Determination of pH_{pzc} of bentonite. The following procedure was employed to determine the pH point zero charge of the bentonite: 500 ml of 0.01 N NaCl solution was prepared and placed in several conical flasks (each contains 50 cm³ of solution). The pH of the solutions in the conical flasks was adjusted between 2 - 10 by adding 0.01 N NaOH or 0.01N HCl solutions. Subsequently, 0.15 g of bentonite clay was added to each conical flask and shaken at 200 rpm for 48 h. Afterward, the pH of the dispersion was measured with digital pH-meter.

FTIR Analysis. The surface functional groups of bentonite and doripenem-loaded bentonite were analyzed using the infrared spectroscopy method (FTIR SHIMAD-ZU 8400S). The FTIR spectra were obtained using KBr methods and recorded in the wavenumber range of 400-4000 cm⁻¹

Adsorption and drug release studies. To study the influence of pH, and time on the amount uptake by the bentonite, the adsorption procedure is as follows: A known amount of doripenem was diluted in 0.01 N of NaCl 34 ution to prepare 200 mg/L of doripenem solution. To study the influence of pH, 50 mL of doripenem solution was added to the iodine flask, and the pH was adjusted using sodium hydroxide or hydrochloric acid solution (between 2 to 10). Subsequently, 0.5 gram of bentonite was added to the solution, and the flask was placed in a shaking water bath and shaking at 200 RPM for 12 h at 30°C. After the adsorption process completed, the solid was separated from the liquid by centrifugation. The drug content in the supernatant (solution) was determined using spectrophotometer UV-Vis at 12 ximum wavelength of 290 nm.

To study the effect of time on the doripenem adsorption on bentonite (adsorption kinetic), the procedure is as follows: doripenem solution with the concentration of 200 mg/L was distributed in a series of iodine flasks (each flask filled with 50 ml of solutions). 0.5 g of bentonite was added to each iodine flask and shaken for 5 min. The iodine flasks were then placed in thermal controller shaking water bath and shaken at 200 RPM and 30°C. At a certain interval of time, one of the iodine flasks was taken from the system, the solid was removed from the solution by centrifugation, and the remaining doripenem in the solution was measured using a spectrophotometer. 13 e amount of doripenem adsorbed onto bentonite at time t (q_t) was determined by the following equation

$$q_t = \frac{(C_o - C_t)}{m} V$$
 [1]

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Where C_0 and C_t are the initial concentration and the concentration of doripenem at time t, m is the mass of bentonite, and V is the volume of the solution.

A similar procedure to the adsorption kinetics was employed to obtain the adsorption isotherm of doripenem onto bentonite at pH 5. In the adsorption isotherm experiment, the amount of bentonite was varied from 0.1 gram to 1.0 gram. The time of the system (doripenem – bentonite) to reach equilibrium 33s around 10 h, and 12 h was taken as the time for the adsorption experiment. The amount of doripenem adsorbed onto the bentonite at equilibrium condition (q_e) was calculated according to the following equation:

$$q_e = \frac{(C_o - C_e)}{m} V$$
 [2]

where C_e is the equilibrium concentration.

The effect of initial pH for the doripenem adsorption by bentonite was studied at a range of pH 2 – 10 (initial concentration of 200 ppm, at 37°C for 48 h). The pH was adjusted at 0.1 N of HCl or NaOH scattion. After equilibrium, the adsorbent was separated using a centrifuge, and the filtrate was analyzed using a Shimadzu UV/Vis-1700 spectrophotometer. The doripenem release study was performed at pH 3 and pH 7. Phosphate buffer solution was employed as the simulated body fluid solution (regulated the pH as well). The release experiment was conducted for 24 h.

Microbial growth inhibition study. Staphylococcus aur 29 was grown in nutrient broth at aerobic condition and temperature of 37°C. The well-diffusion method was used to examine the effect of the antibacterial effects of the bentonite-doripenem composite. Doripenem load was 22 mg/g. Nutrient agar was employed as the media to test the ability of bentonite-doripenem composite to inhibit the growth of Staphylococcus aureus. 1 mL of S. aureus was added to each plate of nutrient agar. The hole was made in the plates were incubated at aerobic condition at 37°C for 24 h. The clear area around the well showed inhibition of the growth of the S. aureus, and the diameter of the clear area was measured.

Results and discussion

Point of Zero Charges (pH_{pzc})

Point of zero charges is the point where the adsorbents have zero potential charges on its surface. The surface charges of clay mineral may be changing because of the presence of groups with a net charge. Below the value of point zero charges, the surface positively charged; beyond the value, it negatively charged. So normally, it is always easier to adsorb a cation on a negatively charged surface and an anion on positively charged surface.

Additionally, a cation often complexed with ligands, some of them being possibly negatively charged. Therefore, in such a case, the cation is, in fact, a negative complex, which may adsorb very well on a positively charged surface. The point of

zero charges of purified bentonite was 3.1. The permanent negative charge of the bentonites is the main contribution to the net charge of mineral.

Characteristics of doripenem-clay

Figure 1 depicts the FTIR spectra of bentonite and bentonite-doripenem composite. Stretching vibrations of functional group C-N bonds in doripenem is at 1039 cm⁻¹. Bending vibration of C-H bond in these ring is located at 1268 cm⁻¹. N–H stretching (sulfonamide group) is located at 1500-1600 cm⁻¹. Carbonyl group stretching (carboxylic acid group) can be seen at 1715 cm⁻¹. The stretching of the alcoholic hydroxyl group is located at 3255 cm⁻¹. N – H stretching (pyrrolidinyl group) also can be seen at 3393 cm⁻¹. The Si-O group which is a characteristic of the bentonite structure is located at 1050 cm⁻¹. Meanwhile, the spectrum analysis of doripenem antibiotics using FTIR cannot be done because the antibiotic doripenem is a liquid form and FTIR analysis can only analyze the solid material.

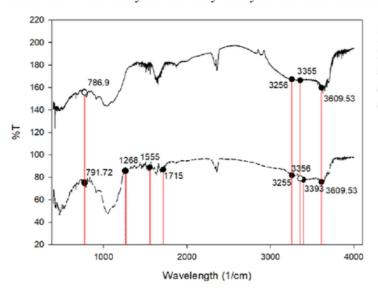
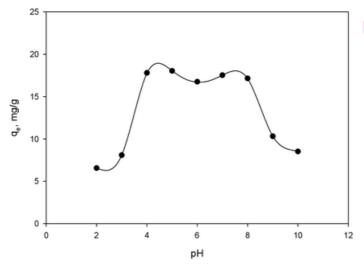


Figure 1
The FTIR Spectra of
bentonite (solid line) and
doripenem - bentonite
composite (dashed line).

Adsorption studies

pH has a significant influence on the adsorption of doripenem as seen in Figure 2. The adsorption of doripenem by bentonite is essentially constant at pH between 4 to 8 and above pH 8 the uptake of doripenem decrease. By increasing pH, the uptake of doripenem by bentonite decrease as indicated in Figure 2. As the member of β-lactam antibiotic group, doripenem contains different ionizable parts either as acidic or basic groups. Doripenem is ampholyte compound; it can exist in three possible forms: cationic, neutral, and anionic. The pKa₁ of doripenem is 2.476 while the pKa₂ is 7.902 (Demiralay et al 2014). At the solution pH below 2.5, it will present in the cationic form, while above 7.90, it will exist in anionic form. The doripenem behaves as zwitterions between pH 2.5 to 7.9.



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As mentioned in the previous section, the pHpzc of the bentonite was 3.1, below pHpzc, the bentonite positively charged, while above pHpzc this clay material negatively charged. At pH below 4, the repulsive interaction between positively charged of bentonite surface and cationic form of doripenem occurred. Therefore, less uptake of doripenem is observed as seen in Figure 2. Similar behavior was observed at pH above 8, the uptake of doripenem decrease with increasing pH of the solution due to the repulsive interaction between the negatively charged of the surface of the bentonite and anionic form of doripenem. At pH 4 to 8, the adsorption of doripenem onto the surface of bentonite possibly due to van der Waals interaction between the zwitterions form of doripenem and 2:1 bentonite layers namely tetrahedral and octahedral. The presence of silanol group from bentonite is the potential binding sites for doripenem chemisorption.

The adsorption kinetics experimental data of doripenem onto bentonite is depicted in Figure 3. From this figure, it can be seen that ad 6 rption of doripenem reaches equilibrium condition after 10 h. The Lagergren pseudo-21st-order (Lagergren 1898) and pseudo-second-order (Blanchard et al. 1984) models were used to correlate the experimental kinetics data. The Lagergren pseudo-first-order model has the mathematical form as follows:

$$q_t = q_e [(1 - \exp(-k_l t))]$$
 [3]

While the pseudo-second-order has the following form:

$$q_e = \frac{Q_2 k_2 t}{q_2 k_2 t} \tag{4}$$

Where k_1^{20} and k_2 are rate constant for Lagergren pseudo-first-order and pseudo-second-order, respectively. The plots of both models on experimental kinetics data are also given in Figure 3, while the parameters obtained from the fitting of the data given in Table 1.

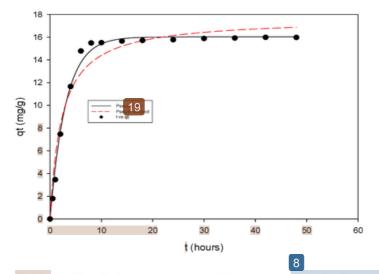


Figure 3
The kinetics of adsorption of doripenem onto bentonite.

Table 1. The fitting parameters of Lagergren pseudo-first-order and pseudo-second-order models for the adsorption effortinem onto bentonite

Concentration	Pseudo First Order			Pseudo Second Order		
(mg/L)	$\mathbf{k_1}$	qe (mg/g)	\mathbb{R}^2	\mathbf{k}_2	Qe (mg/g)	\mathbb{R}^2
200	0.3261	16.0263	0.9935	0.4403	7.7799	0.9819

Both kinetic models show a good fit (R² greater than 0.90) which can refer to Figure 3 and Table 1. The adsor 25 n isotherm of doripenem onto bentonite is given in Figure 4. Well known adsorption isotherm equations, Langmuir and Freundlich models were employed to correlate the experimental adsorption data. Langmuir model has developed almost one decade ago. This equation was derived based on the assump 3 ns: the surface of the adsorbent is homogeneous, the adsorption that occurs on the surface is localized, and each adsorption site can only accommodat 32 ne molecule of adsorbate. Based on those assumptions, the mathematics form of the Langmuir equation can be written as

$$q_e = q_{max} - \frac{K_L C_e}{I + K_L C_e}$$
 [5]

where K_L and q_{max} are the Langmuir parameters which represent the adsorption affinity and the adsorption capacity of the adsorbent, respectively.

Freundlich eq 3 ion is an empirical equation that can represent the adsorption of many systems. This equation is one among the earliest empirical equations employed to predict adsorption equilibrium data. The Freundlich equation can be written in the following form:

$$q_e = K_F C_e^{1/n} \tag{6}$$

where K_F (mg/g) and n are the Freundlich constants. The parameter K_F indicates the Freundlich adsorption capacity while the parameter n characterizes the heterogeneity of the system. The parameter n usually greater than unity.

The experimental adsorption data with the theoretical plots of Langmuir and Freundlich adsorption equations are given in Figure 4. The parameters of Langmuir and Freundlich equations are summarized in Table 2.

 Table 2. The fitting parameters of Langmuir and Freundlich equations for the adsorption of doripenem onto bentonite

Concentration	31 Langmuir equation			Freundlich equation		
(mg/L)	$\mathbf{K_L}$	Q_{max}	\mathbb{R}^2	$K_{\mathbf{f}}$	n	\mathbb{R}^2
200	0.0193	31.39	0.9491	2.67	2.28	0.9159
					23	

From the value of R² and visual look of Figure 4, it is clear that the Langmuir equation can predict the experimental data better than Freundlich equation. In both cases, a good fit is observed.

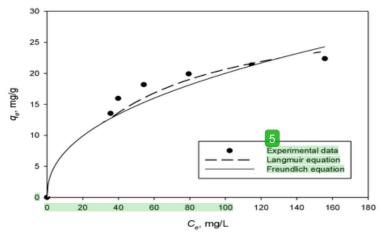


Figure 4
Adsorption isotherm
of doripenem on
bentonite

In many cases, Langmuir equation gives better performance than Freundlich model due to the presence of limit saturation capacity at high concentration and Henry law at low concentration.

Drug release studies

Drug release study of loaded doripenem from bentonite was carried out in buffer phosphate solutions at pH 3 and pH 7 and temperatures of 37°C. Figure 5 depicts the release profiles of doripenem from bentonite. From this figure, it can be seen that pH 3 gave better release amount of doripenem than pH 7. The zero charge point of bentonite is 3.1 thus it is expected that under these conditions the surface charge is not determinant of this behavior, and some of the attached doripenem was released to the buffer solution due to repulsion forces of the bentonite surface.

Microbiological testing

The most common action of antibiotics to inhibit the growth of bacteria is the inhibition of cell wall synthesis. Antibiotics inhibit cell wall synthesis because most of the bacteria have peptidoglycan-based cell walls.

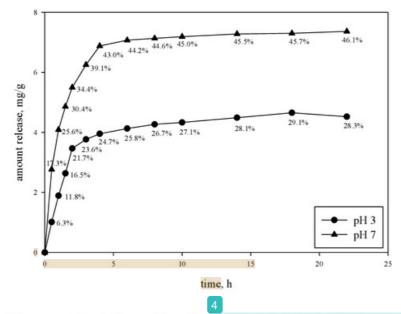


Figure 5
The release profile of doripener 30 om bentonite at pH 3 and pH 7.

The growth of the cell walls is prevented by inhibiting peptidoglycan synthesis. Thus, the antibiotics only work for actively growing bacteria.

One of the methods to examine the effectiveness of antibiotics is the well-diffusion method. The clear zone around the well showed the effectiveness of the antibiotic composite. Figure 6 shows some pictures of the effectiveness of doripenembentonite composite that inhibit the growth of *Staphylococcus aureus*.



Figure 6. The effectiveness of doripenem-bentonite composite that inhibits the growth of Staphylococcus aureus.

The microbiological test gresults indicate that doripenem-bentonite composite has potential application to inhibit the growth of *Staphylococcus aureus* and other bacteria.

Conclusion

Doripenem-bentonite composite has been synthesized in this study. The effect of pH and adsorption time on the amount of doripenem adsorbed by bentonite has also been studied. The adsorption of doripenem onto bentonite mostly due to van der Waals interaction between doripenem molecules and 2:1 layer spacing of bentonite. The pH of the solution influenced the adsorption process, below pH 4 and above pH 8, the repulsive interaction between the surface of bentonite and the pripenem ions occurred resulting low uptake of doripenem by bentonite. The pseudo-first-order model could represent the adsorption kinetic data better than the pseudo-second-order model. Two well-known adsorption models, Langmuir and Freundlich, were employed to correlate the adsorption isotherm data, and Langmuir could give a better result than Freundlich. The release of doripenem from the bentonite was studied at pH 3 and 7, and higher doripenem release was observed at pH 3. The microbiological testing results indicate that doripenem-bentonite composite has potential application to inhibit the growth of *Staphylococcus aureus* and other bacteria.

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