Characterization and Antibacterial Activity of Streptomycin Antibiotic Loaded Organo-Kaolinite

Characterization and Antibacterial Activity of Streptomycin Antibiotic Loaded Organo-Kaolinite

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Abstract. Antibiotics are medicines used against bacterial infection. In spite of its significance to kill bacteria, it also shows a problem with antibiotics resistance that makes it less effective to kill bacteria. Therefore, an improvement for the antibacterial agent is needed to inhibit bacteria growth and infections. Organo-kaolinite was selected in this study to act as a carrier system to improve antibacterial agent immobilization and increase the effectiveness of the antibiotic activity. Organo-kaolinite was prepared using cationic surfactant hexadecyltrimethylammonium (HDTMA) and it was adsorbed with different concentrations of streptomycin. The organo-kaolinite and streptomycin-organo-kaolinite were characterized by Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD). The characterization results showed that the attachment of cationic surfactant molecules and the adsorption of streptomycin on kaolinite surfaces did not affect the structure and the original morphology of the kaolinite. The antibacterial assay of the samples was carried out against Gram negative bacteria (*Escherichia coli* ATCC 11229) and Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212) through disk diffusion technique (DDT). Based on the antibacterial assay results, streptomycin-organo-kaolinite showed better antibacterial activity compared to organo-kaolinite. This study revealed that the adsorption of streptomycin on organo-kaolinite showed a significant effect on killing bacteria and significantly increased its antibacterial activity compared to organo-kaolinite.

Keywords: Organo-kaolinite, streptomycin, antibacterial agent

1. Introduction

Antibiotics are medicines that fight bacterial infections, either by killing the invading bacteria or weakening them. It will help the immune system to fight and kill them more rapidly. Besides, their functions in killing bacteria, it shows a problem related to bacterial resistance which makes the antibiotics less effective in treating bacterial infections. Evaluation and adaptation are one of the ways for the bacteria to overcome antibiotics [1]. The bacteria will change the permeability of its membrane to prevent antibiotic from opening up its cell membrane. Therefore, an improvement for the

antibacterial agent is needed to inhibit bacteria growth and infections which can lead to more severe diseases. One approach is by combining two or more antibacterial compounds on a carrier system to synergize the antibacterial effects.

Kaolinite is a harmful charge clay mineral with a soft consistency and earthy texture. It consists of 1:1 layer clay composed of a repeating layer of an aluminum octahedral (O) sheet and a silicon tetrahedral (T) sheet [2]. Kaolinite can be loaded with hexadecyltrimethylammonium (HDTMA) forming inorganic-organic materials that make it a suitable candidate for immobilization of antibiotic molecules. Organo-clay is a type of modified clay that has been attached or immobilized with surfactant molecules such as from the group of cationic surfactant quaternary ammonium compounds namely cetylpyridinium bromide (CPB) or HDTMA bromide [3–5]. An organic-rich layer will be formed on the surface layer of the clay when the immobilizations of surfactant molecules occur on the framework structure of the clay. This process of immobilization will upgrade its functionality and cause changes to some of the physiochemical properties of the clay [6]. Therefore, this study aimed to prepare and characterize streptomycin adsorbed on organo-kaolinite and study its antibacterial activity to enhance the antibacterial activity of streptomycin antibiotic.

2. Methods

Kaolinite was purchased from Kaolin (M) Sdn. Bhd located at Tapah, Perak, Malaysia. The kaolinite was modified by using surfactant HDTMA-Br 4.0 mM. For sample preparation, four types of samples had been prepared namely kaolinite, kaolinite adsorbed HDTMA-Br (4.0 mM) known as organo-kaolinite, organo-kaolinite adsorbed streptomycin and kaolinite adsorbed streptomycin with different concentrations of streptomycin. Table 1 gives the list of sample name and their respective description and condition in this study.

Table 1: List of samples and their description

Sample	Description
RK	Raw Kaolinite
STR	Streptomycin
SK	Streptomycin + Kaolinite
HK	Raw kaolinite + HDTMA-Br (4.0 mM) or also mentioned as organo kaolinite
SHK50	HK + STR 50 mg/L
SHK100	HK + STR 100 mg/L
SHK200	HK + STR 200 mg/L
SHK400	HK + STR 400 mg/L
SK50	K + STR 50mg/L
SK100	K+ STR 100mg/L
SK200	K + STR 200mg/L
SK400	K + STR 400mg/L

All samples were then analyzed by using Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) for the detection of organic compounds attached on the kaolinite and their structural analysis. For FTIR analysis, an Attenuated Total Reflection (ATR) technique of FTIR spectrophotometer (Thermo Fisher Scientific Nicolet iS5 assembled with OMNIC™ software) and XRD analysis on Bruker AXS GmbH, German machine were used in this study. The second stage was the antibacterial assay to study the antibacterial activity of the samples. The antibacterial activity of the samples was evaluated against both Gram-positive and Gram-negative bacteria which involved *Escherichia coli* ATCC 11229 (EC) and *Enterococcus faecalis* ATCC 29212 (EN), respectively. For this assay, disc diffusion technique (DDT) was employed to evaluate the antibacterial activity of the prepared samples.

3. Results and Discussion

In this study, the FTIR spectra of raw kaolinite and organo-kaolinite were analyzed to determine the changes in the chemical structures of kaolinite after modification with a cationic surfactant, HDTMA-Br, and antibiotic STR. In addition, it was used to detect the presence of HDTMA molecules and STR on the modified kaolinite. Figure 1 shows FTIR spectra of raw kaolinite and organo-kaolinite before and after adsorption of streptomycin (STR), respectively.

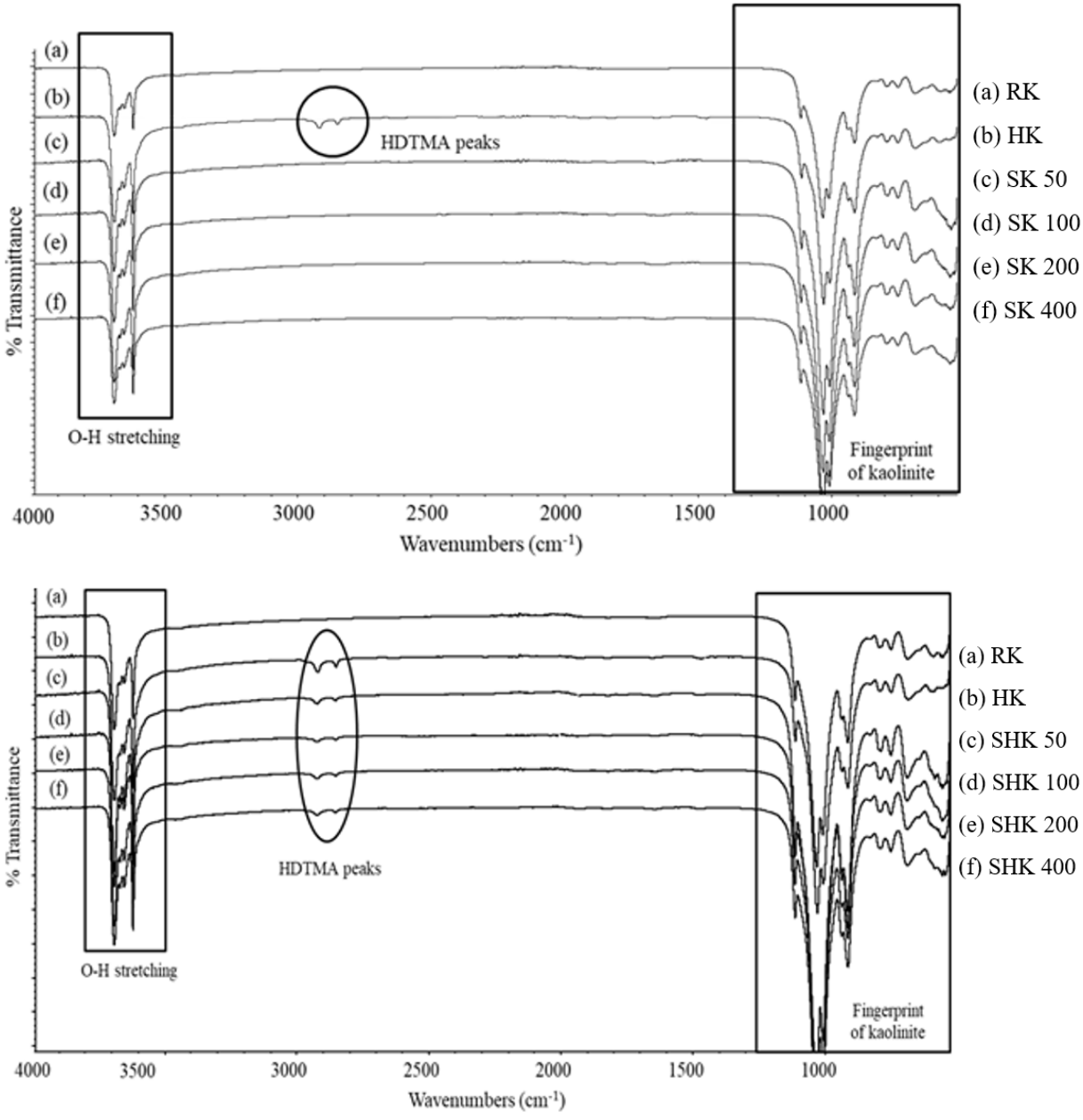

Figure 1. FTIR spectra of the samples

In the FTIR spectra of the samples, the bands at 1113, 1030, and 1006 cm^{-1} corresponded to the Si-O-Si stretching vibration of the tetrahedral sheet while the band at 912 cm^{-1} represented Al-O stretching of kaolinite structure [7]. Two new peaks can be seen at 2923 and 2836 cm^{-1}. The two peaks represented the presence of cationic surfactant HDTMA molecule on kaolinite structure. These peaks were absent in the FTIR spectra of raw kaolinite. The peaks formation was attributed to symmetric and

the latter for asymmetric stretching vibration of C-H in the alkyl chain of HDTMA-Br [8]. These peaks showed that HDTMA-Br molecules were attached on the kaolinite. The absence of multiple functional groups often overlapped with each other, and this aspect of IR spectra made it difficult to determine some specific molecular structures. The adsorption of STR onto organo-kaolinite and on raw kaolinite did not show any significant additional peaks due to its similar functional groups that were overlapped in their peaks in the FTIR spectra of raw kaolinite and organo-kaolinite.

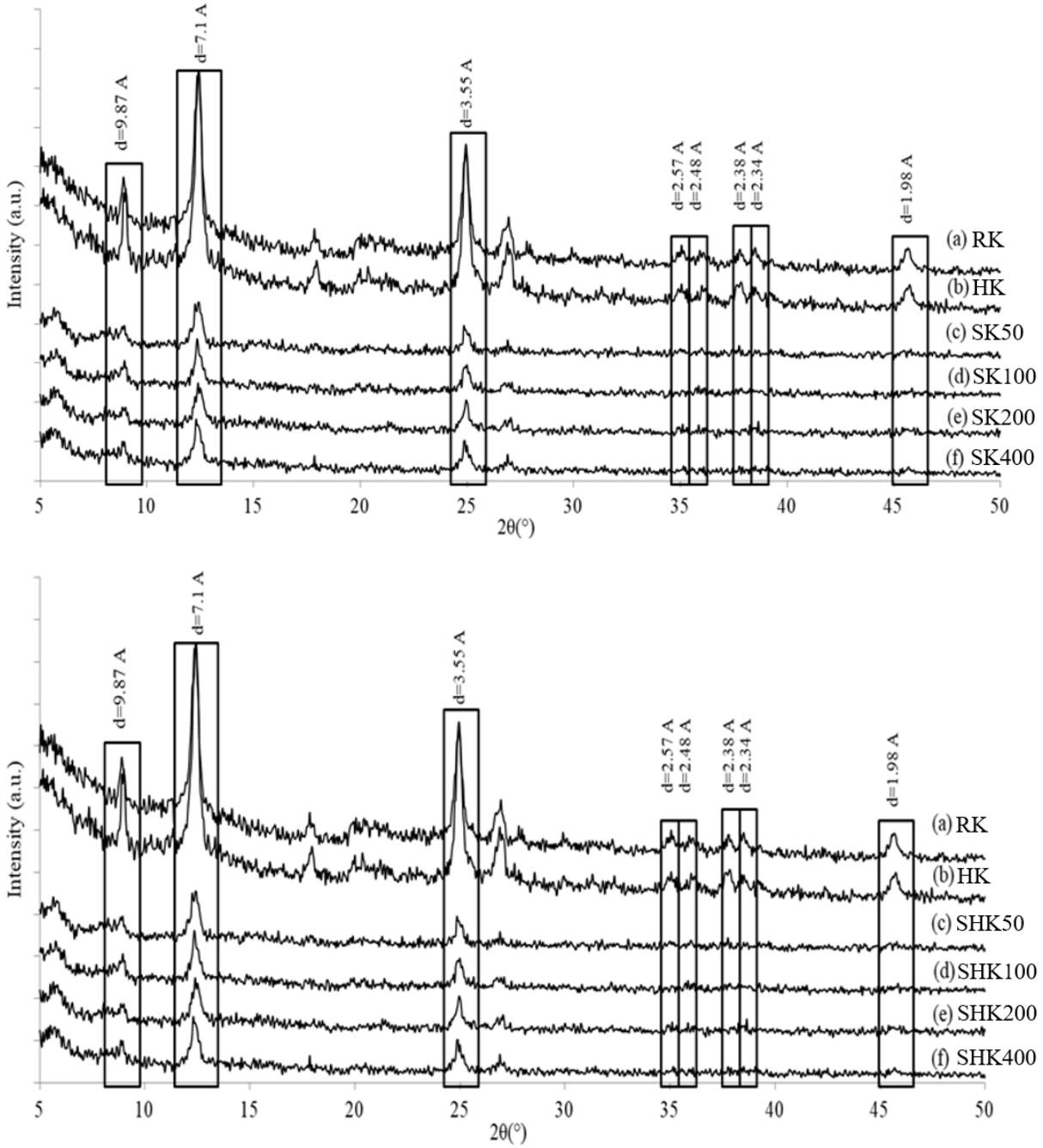

Figure 2. X-ray diffractogram of the samples.

Figure 2 shows the XRD patterns of raw kaolinite and organo-kaolinite adsorbed streptomycin. The diffractogram displays important peaks from 5º to 50º which proved that the samples were in crystalline form. Based on the diffractograms, an intense reflection of d-spacing at 7.1 Å represented

kaolinite structure. The XRD pattern of the kaolinite also displayed strong peaks at 3.55 Å that could be attributed to kaolinite d001 and d002 reflections and peaks at 4.45, 2.57, 2.48, 2.38 and 1.98 Å were all consistent with that of kaolinite. Illite is a common impurity found in kaolinite which showed a major peak at 9.87 Å. The XRD patterns of organo-kaolinite and kaolinite before and after the adsorption of streptomycin (STR) showed the same pattern with raw kaolinite as there were no significant changes in the XRD patterns. This case indicated that the HDTMA-Br and streptomycin molecules were intercalated on the kaolinite surface [5]. In addition, the interlayer spaces will be expanded if kaolinite is intercalated with the intercalation guests [9]. As a conclusion, the aluminum silicate framework of modified kaolinite did not change, disrupt or damage after modification and the structural stability of the kaolinite could be maintained.

For the antibacterial activity of the samples, Figure 3 shows the images of the plate from DDT for raw kaolinite and modified kaolinite and Table 2 shows the inhibition zone (in cm) values from the DDT results. Based on Figure 3, raw kaolinite (RK) showed no inhibition zone for both types of bacteria indicating that RK alone had no antibacterial activity. This result proved that the natural clay minerals showed no antibacterial effect unless some antibacterial agents were adsorbed or intercalated on the clay [10]. Organo-kaolinite (HK) before and after adsorption of streptomycin showed very clear inhibition zones around the pellet. These results indicated that HK and SHK samples with different concentrations of streptomycin were effective against both Gram negative and Gram-positive bacteria due to the loading of HDTMA and STR inside the kaolinite.

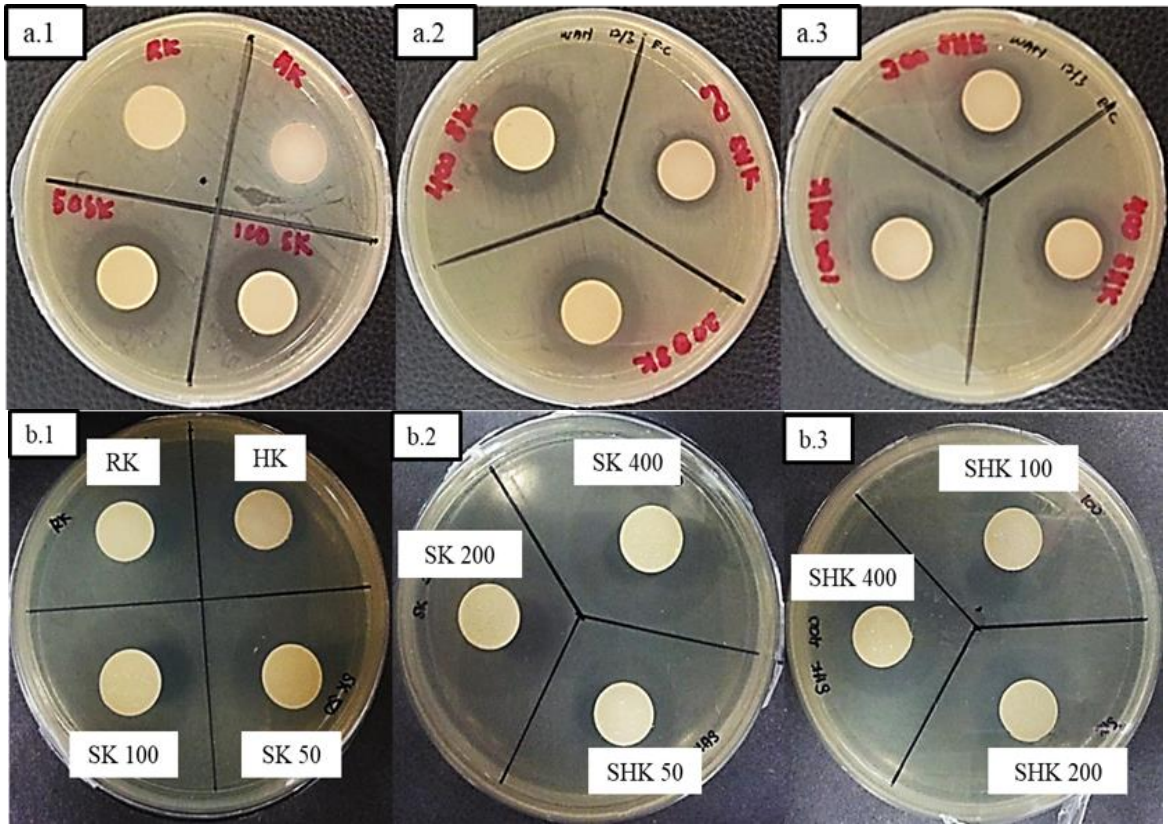

Figure 3. Images of plates from DDT for raw kaolinite and organo kaolinite for bacteria (a) *E. coli* and (b) *E. faecalis.*

Table 2. Inhibition zone (in cm) values and the standard deviation from the DDT

Bacteria and Samples	Bacteria strain	
	Gram-positive *E. faecalis*	Gram-negative *E. coli*
Distilled water	-	-
RK	-	-
HK	2.20±0.265	-
SK 50	3.00±0.520	2.17±0.058
SK100	3.23±0.351	2.37±0.115
SK 200	3.47±0.252	2.43±0.058
SK 400	3.57±0.252	2.50±0.100
SHK 50	2.17±0.306	1.73±0.058
SHK 100	2.37±0.404	1.83±0.058
SHK 200	2.40±0.436	1.90±0.000
SHK 400	2.60±0.436	2.00±0.000

The presence of inhibition zone proved the diffusion of HDTMA and STR molecules from the kaolinite into the agar. Besides, the increment of the diameter with the increase in STR concentrations showed that the STR molecules were successfully adsorbed by organo-kaolinite. The mechanisms of the bacteria inhibition could be explained by the electrostatic attraction between positively charged HDTMA cation interacts with the negatively charged bacterial cells. HDTMA molecules attack bacteria cell wall and disrupt the integrity of the cell membrane and thus, it causes leakage to the cytoplasm. This case causes damage to the bacteria system and enzyme inhibition of the organisms. As a result, this case causes bacterial cell death. The result showed that *E. faecalis* was more susceptible than *E. coli* because *E. coli* a have more complex system so that the antibacterial agents are difficult to penetrate to *E. coli*.

4. Conclusion

Based on the results from FTIR and XRD, it revealed that the original interlayer structure of raw kaolinite remained unchanged after modification with a cationic surfactant (HDTMA-Br) and adsorption of STR. From the characterization result and antibacterial assay, it can be concluded that HDTMA-Br and STR molecules were successfully adsorbed onto kaolinite surface. The raw kaolinite and organo kaolinite with and without adsorption of STR were tested against Gram negative bacteria (*E. coli* ATCC 11229) and Gram-positive bacteria (*E. faecalis* ATCC 29212) using disc diffusion technique (DDT). Organo-kaolinite with the highest concentration of STR which was 400 mg/L showed the highest antibacterial activity by showing the biggest inhibition zone for both types of bacteria. As a conclusion, organo-kaolinite with the adsorption of STR can be a good antibacterial agent to inhibit the bacterial growth for Gram positive bacteria.

Acknowledgements

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