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RESEARCH ARTICLE

Adsorption of gentamicin on surfactant-kaolinite and its antibacterial activity

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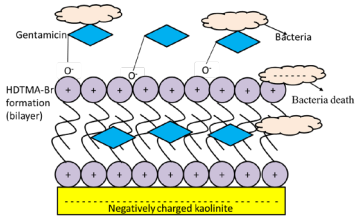
Graphical abstract



Abstract

Kaolinite is a common component of soil. Negatively-charged kaolinite can act as an adsorbent material and it has the ability to adsorb antimicrobial agents. In this study, local natural kaolinite was used to adsorb gentamicin and cationic surfactant molecules. Gentamicin-loaded surfactant-kaolinite (GSK) was prepared firstly by the attachment of cationic surfactant 4.0 mM hexadecyltrimethyl ammonium (HDTMA) on raw kaolinite to produce surfactant-kaolinite (SK), which was then loaded with gentamicin sulphate (50 and 200 mg/L) to yield GSK. Gentamicin-loaded kaolinite (GK) was also prepared and compared. All samples were characterised by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and CHNS elemental analysis. The characterisation results proved that the framework structure of kaolinite was not disrupted after modification with antimicrobial agents. The antibacterial activity of the samples was tested against Gram-negative *Escherichia coli* (ATCC 11229) and Gram-positive *Enterococcus faecalis* (ATCC 29212) through disc diffusion technique (DDT). Based on the technique, raw kaolinite did not exhibit antibacterial activity but showed antibacterial activity when HDTMA and/or gentamicin was loaded on kaolinite. In addition, GSK showed better antibacterial activity compared to GK and performed better on Gram-positive bacteria compared to Gram-negative bacteria. As a conclusion, immobilisation of HDTMA on kaolinite proved that kaolinite can act as an adsorbent to adsorb antibiotics and it has the potential to be developed as an enhanced antimicrobial agent.

***Keywords*:** Antibacterial, gentamicin, kaolinite

INTRODUCTION

In recent years, aminoglycoside antibiotics have been extensively used in medicine, veterinary, and agriculture fields due to their broad antibacterial spectrum, high efficiency, and cost effective (Wu *et al.*, 2002; Xu *et al.*, 2017). Gentamicin, which is an example of aminoglycoside antibiotic, is proven in healing infections and simultaneously improving the growth of livestock farming (Kemper, 2008). Ojdana *et al.* (2018) and Rapacz-Kmita *et al.* (2017) stated that gentamicin suppresses or kills a broad range of Gram-negative bacteria and it is effective against *Staphylococcus, Streptococcus*, and *Enterococcus*, which are major pathogenic microorganisms that can cause infective endocarditis. Likewise, Nakashima *et al.* (2000) concluded that the application of gentamicin injections can treat 90%–100% of vertigo due to Meniere's disease. On the other hand, in terms of agricultural industry, gentamicin is substantially used to combat diseases and acted as a prophylactic agent to enhance the growth of livestock (Rapacz-Kmita *et al.*, 2017). Hence, for this reason, farmers often apply gentamicin antibiotic for the livestock to avoid chronic pathogenic contamination. However, the extensive use of gentamicin exhibits consequences to other living organisms and the environment. Gentamicin is partially metabolised by animals; hence, it is mostly discarded due to its function as bioactive substances. Therefore, the increasing volume of gentamicin released to the environment can result in environmental pollution and also potential problems with the increasing bacterial resistance to gentamicin. One approach that can be applied to overcome this problem is by immobilising the antibiotic compound onto suitable materials such as kaolinite clay.

Kaolinite is a type of clay and a common component of soils and sediments. Kaolinite is an example of 1:1-layer structure type clay that has 0.72 nm basal spacing with the possible composition of $Al_2Si_2O_5(OH)_4$ (Young and Hewat, 1988). Researchers use kaolinite as an adsorbent material for the adsorption of various antibacterial and antibiotic compounds such as chlortetracycline, tylosin, sulfamethazine, quinolone, and chlorhexidine (Essington *et al.*, 2010; Wu *et al.*, 2013; Jou and Malek, 2016). Previous studies showed that many clay minerals including kaolinite has a high affinity for antibiotics such as sulphonamide (Malek *et al.*, 2018) and tetracycline (Wu *et al.*, 2013), which may result in the immobilisation of antibiotics on clay. However, kaolinite has low adsorption capacity due to the absent of an interlayer space within the kaolinite structure (Essington *et al.*, 2010). Nevertheless, the adsorption capability of kaolinite can be enhanced by immobilising quaternary ammonium compound (QAC) cationic surfactant molecules such as hexadecyltrimethylammonium bromide (HDTMA) onto its surface to produce organo-clay (Aziz *et al.*, 2018). Moreover, surfactant-modified kaolinite has been discovered as an antibacterial agent by previous studies (Saad *et al.*, 2016; Malek and Ramli, 2015).

QAC such as HDTMA consists of hydrophobic tails and hydrophilic head groups (Fig. 1). Theoretically, the hydrocarbon

group replaces one of the four hydrogen (H) molecules that directly bonded to one nitrogen (N) molecule in HDTMA-Br. According to Özdemir *et al.* (2010), QAC exhibits antibacterial properties. This is supported by Wu *et al.*, (2011) where antibacterial activity is presented for montmorillonite loaded with tetradecyltributyl phosphonium bromide. Moreover, surfactant tends to bind on the negative charge surface of clay and form a monolayer of surfactants with the tails pointing outward (Özdemir *et al.*, 2010). As the concentration of surfactant is increased, it will lead to the bilayer formation of surfactant molecules on the surface. The formation will occur when the addition of surfactant exceeds the cationic exchange capacity (CEC) of the clay, such as kaolinite (Malek and Ramli, 2015). Therefore, the bilayer formation of surfactant will trigger some changes on kaolinite surface properties and enable the adsorption of other organic compounds onto the clay surface, such as aminoglycoside antibiotic compounds.

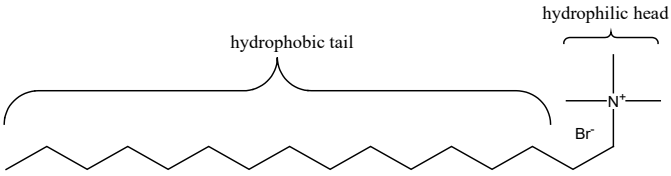


Fig. 1 Formula structure of HDTMA-Br with hydrophobic and hydrophilic regions.

The purpose of the present study was to prepare and characterise kaolinite, surfactant-kaolinite (HDTMA-Br modified kaolinite), and gentamicin-surfactant-kaolinite. Their antibacterial activity was studied towards Gram-negative (i.e., *Escherichia coli*) and Gram-positive (i.e., *Enterococcus faecalis*) bacteria in order to study the feasibility of using local raw kaolinite as a carrier system for antibacterial and antibiotic compounds.

EXPERIMENTAL

Materials

The starting materials, which were gentamicin (G) in the form of gentamicin sulphate ($C_{21}H_{43}N_5O_7.H_2SO_4$, Sigma-Aldrich Co LLC), raw local kaolinite (RK, Kaolin (M) Sdn. Bhd.), and HDTMA-Br (Sigma-Aldrich Co LLC), were used in the preparation of materials.

Preparation of materials

For the preparation of surfactant-kaolinite (SK), a stock solution of HDTMA-Br (4.0 mM) was initially prepared by dissolving 1.458 g of HDTMA-Br in 1,000 ml of distilled water, and the solution was stirred thoroughly until it changed from a cloudy to a clear solution. Approximately 2.0 g of RK was weighed and added into 200 ml of HDTMA-Br solution in a beaker. By using a magnetic stirrer, the mixture was stirred for 16 to 17 h. Macherey-Nagel filter paper (125 mm) was used to separate the mixture of solid and liquid. Prior to drying the solid portion, the sample was placed in an oven at 80°C overnight. The dried solid sample was ground into powder form by a mortar and pestle and then sieved. Finally, the sample (SK) was kept in a clean plastic container to be readily used for the adsorption of gentamicin compound. For the preparation of gentamicin-loaded surfactant-kaolinite (GSK), gentamicin solutions (50 and 200 mg/L) were firstly prepared. About 0.5 g of the prepared SK was added into 50 ml of gentamicin solution. The remaining procedures were similar to that of the preparation of SK. Gentamicin-loaded kaolinite (GK) was also prepared based on the same procedures for the preparation of GSK but without the presence of HDTMA. The prepared samples were designated as RK, SK, 50GK, 200GK, 50GSK, and 200GSK, in which 50 and 200 were represented for 50 and 200 mg/L of gentamicin, respectively.

Characterisation

The samples were characterised by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and CHNS analyser. An XRD instrument (Bruker AXS GmBH, Germany) was used for structural identification. The XRD patterns were recorded using Cu-K_α radiation with $\lambda = 1.5406$ Å at 40 kV and 20 mA over a range of $2\theta = 5°$ to 50° with a scanning speed of 0.05° per second. Thermo Fisher Scientific Nicolet iS5 FTIR spectrometer assembled with OMNIC™ software was used to detect the presence of HDTMA-Br and gentamicin in all samples. The samples were analysed for their content of C, H, N and S elements by an elemental analyser (Vario MICRO cube) at University Industry Research Laboratory (UIRL), Universiti Teknologi Malaysia (UTM).

Antibacterial assay

The antibacterial activity of all samples was evaluated against *E. coli* (ATCC 11229) and *E. faecalis* (ATCC 29212) based on disc diffusion technique (DDT). For the evaluation, 0.15 g of each sample was initially pressed into a pellet disc by using a hydraulic press pump at a pressure of 1400 psi. Then, the pellets were kept in a sealed plastic bag for DDT. After that, the bacteria were cultured on a nutrient agar (NA) at 37°C for 24 h. Then, three to five bacteria colonies were transferred in a sterilised 0.9% (w/v) saline solution. The turbidity of the suspension was compared to 0.5 McFarland standard turbidity (1.5×10^8 CFU), in which an adjustment was made until the turbidity of the suspension was equivalent to the standard value. Then, the suspension of the bacteria was spread on Mueller Hilton Agar (MHA) using a sterile cotton swab by rotating the plate every 60° to ensure homogenous bacteria growth. Then, the previously prepared pellet discs were placed on top of the surface of the agar plate. Gentamicin solution (1 mg/ml) that was dipped in a round paper disc was used as a positive control. The diameter of inhibition zone (in cm) was measured using a ruler after incubated at 37°C for 24 h under aerobic condition.

RESULTS AND DISCUSSION

Characterisation of materials

Fig. 2 shows XRD patterns of RK and modified kaolinites. Two intense reflections in the XRD diffractogram were used for the determination of kaolinite structure: d(001) and d(002) basal reflection at 7.1 and 3.6 Å, respectively (Malek and Ramli, 2015). The reflections at 4.45, 2.57, 2.48, 2.38, and 1.98 Å could also be used to determine the kaolinite framework structure (Jou and Malek, 2016). The basal reflection for kaolinite before and after the modification remained the same as it could be considered that the modification of kaolinite with surfactant and gentamicin did not distort the framework structure of kaolinite. Furthermore, the XRD patterns also showed no new peaks formed and no change in peak locations. Hence, it could also be explained that the modification of kaolinite with gentamicin and surfactant occurred at the external surface of kaolinite without affecting kaolinite structure (Malek *et al*., 2015).

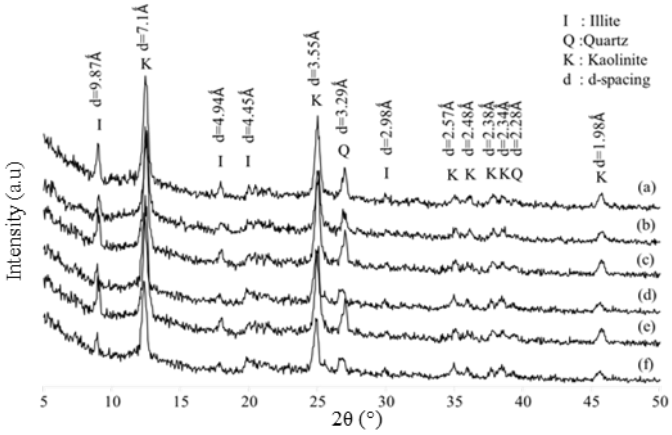


Fig. 2 X-ray powder patterns of (a) RK, (b) SK, (c) 50GK, (d) 200GK, (e) 50GSK, and (f) 200GSK.

The FTIR spectra of all samples are given in Fig. 3. At the O-H stretching region, all of the samples have three significant bands specifically at 3620, 3653 and 3695 cm^{-1} that represented for Al-OH

stretching. Absorption at 3620 cm^{-1} represented the inner hydroxyl groups that centrally located at tetrahedral and octahedral sheets. Pavlukhina *et al.* (2014) stated that the presence of gentamicin can be proven by an increase of peak intensity at 1558 cm^{-1}. However, due to less concentration of gentamicin used, the peak was notably unclear on the FTIR spectra. Si-O stretching in the kaolinite structure could be represented by strong peaks at 1120–1000 cm^{-1} region (Panda *et al*., 2010). Above all, the framework of kaolinite was not disrupted by the modification with HDTMA and gentamicin since there was no obvious change in the FTIR spectra of modified kaolinite compared to raw kaolinite. This could be proven by the same FTIR bands at the fingerprint region (bands below 1500 cm^{-1}) for all samples. However, two additional peaks with very minor intensity at 2920 and 2850 cm^{-1} were observed for kaolinite modified with surfactant (SK and GSK). These peaks represented symmetric and asymmetric vibrations of C-H stretching modes for the hydrocarbon tail of HDTMA-Br adsorbed on kaolinite (Malek and Ramli, 2015). This finding showed that SK and GSK were attached with HDTMA-Br molecules and this could change the surface properties of kaolinite.

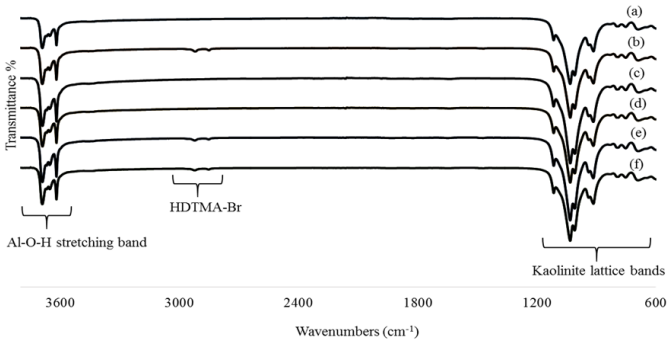


Fig. 3 FTIR spectra of (a) RK, (b) SK, (c) 50GK, (d) 200GK, (e) 50GSK, and (f) 200GSK.

The basic elements of organic compounds including carbon (C), hydrogen (H), nitrogen (N), and sulphur (S) in the samples were detected using CHNS analyser and the data are given in Table 1. The high content of C, H, and N elements in SK, 50GSK and 200GSK was due to the adsorption of HDTMA molecules on kaolinite. The C and H elements were found at the long hydrocarbon chains of HDTMA molecules, whereas N element was found at the amine group (i.e., hydrophilic head) of HDTMA molecules.

Table 1 Elemental chemical analysis (C, H, N, and S) of samples.

Samples	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulphur (%)
RK	0	1.260	0.016	0.066
SK	2.398	1.575	0.154	0.080
50GK	0.007	1.335	0.067	0.087
200GK	0.007	1.339	0.051	0.074
50GSK	1.637	1.410	0.094	0.149
200GSK	1.784	1.495	0.088	0.186

The elemental chemical analysis also showed that the increase of C, H, N, and S elements in 50GK and 200GK was due to the adsorption of gentamicin on kaolinite. Kaolinite did not contain those elements and thus, those elements were attributed from HDTMA and gentamicin molecules immobilised on kaolinite. In short, the modification of HDTMA and gentamicin on kaolinite resulted in the increase of C, H, N, and S elements in modified kaolinites.

Antibacterial assay

The images of inhibition zones formed around the sample pellets on the inoculated MHA surface containing bacteria are shown in Fig. 4 and the inhibition zone values (in cm) are given in Table 2.

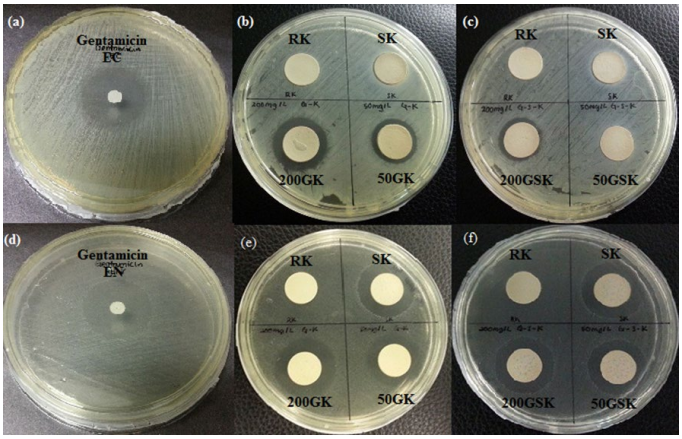


Fig. 4 Images of inhibition zones around the gentamicin discs on the MHA containing (a) *E. coli* and (d) *E. faecalis*, images of inhibition zones around the RK, SK, 50GK, and 200GK pellets on the MHA containing (b) *E. coli* and (e) *E. faecalis*, and images of inhibition zones around the RK, SK, 50GSK, and 200GSK pellets on the MHA containing (c) *E. coli* and (f) *E. faecalis*.

Table 2 Inhibition zone values (in cm) from DDT.

Samples	*E. faecalis*	*E. coli*
RK	0	0
SK	1.00	0.07
50GK	0.70	0.33
200GK	0.90	0.57
50GSK	0.73	0.12
200GSK	0.83	0.20

As expected, RK revealed no antibacterial activity based on the results in Fig. 4 and Table 1 since there was an absence of an inhibition zone around the RK pellets. However, RK was able to kill the bacteria when intercalated with antimicrobial agents (Rapacz-Kmita *et al.*, 2017). On the other hand, Fig. 4 illustrates a clear inhibition zone around the pellets when kaolinite was immobilised with gentamicin or/and surfactant. The biggest inhibition zone was showed by SK for *E. faecalis* due to the nature of HDTMA molecules itself where the amphiphilic part was selectively targeted the cell wall of bacteria, thus producing acute toxicity that would penetrate the cytoplasmic membrane of bacteria (Block, 2001). However, SK was slightly affected by *E. coli* (Gram-negative) compared to *E. faecalis* (Gram-positive), possibly due to the complexity of Gram-negative bacterial membrane. In contrast, GK demonstrated slightly decreased inhibition zone than SK. However, the inhibition zone was increased slightly when higher concentration of gentamicin was applied. This was suggested by a possible antibacterial mechanism of gentamicin in which the gentamicin could interfere with protein synthesis by binding to A site of rRNA and therefore, inhibiting protein synthesis in the bacteria (Moazed and Noller, 1987; Woodcock *et al.*, 1991). GSK also showed an average inhibition zone, similar to SK. Nevertheless, the inhibition zone of GSK was increased when a higher concentration of HDTMA and gentamicin adsorbed on kaolinite. Supposedly, GSK should demonstrate the highest inhibition zone or antibacterial activity among those samples because two antibacterial agents were loaded on kaolinite. However, this might be due to desorption of one or both antibacterial agents from the active carrier system (kaolinite) during the preparation process that led to an insignificant inhibition zone. In brief, this result indicated that SK, GK, and GSK showed antibacterial activity against Gram-positive and Gram-negative bacteria. This also proved that the loadings of surfactant and gentamicin compounds were successfully achieved within the RK surface.

The antibacterial activity of modified kaolinites was more effective when a higher concentration of gentamicin was loaded onto RK and SK. More antibacterial agents within RK could penetrate into the cell membrane, resulting in the leakage of intracellular ions and eventually leading to the death of bacteria (Balouiri *et al.*, 2016; Ojdana *et al.*, 2018; Rapacz-Kmita *et al.*, 2017). In addition, modified kaolinite was more effective in killing Gram-positive bacteria than Gram-negative bacteria because Gram-positive bacteria have a less complicated cell membrane than Gram-negative bacteria. Therefore, Gram-positive bacteria were more susceptible to the studied materials compared to Gram-negative bacteria (Balouiri *et al.*, 2016).

CONCLUSION

In conclusion, HDTMA-Br and gentamicin compounds were successfully loaded and immobilised on the surface of kaolinite clay. The characterisation of GSK revealed that the adsorption of surfactant (HDTMA-Br) and gentamicin on kaolinite did not distort the structure of kaolinite. The adsorption of surfactant and gentamicin influenced the antibacterial activity of GSK, where SK exhibited a slightly higher antibacterial activity compared to GSK on Gram-positive bacteria than Gram-negative bacteria by producing bigger inhibition zones based on DDT.

In addition, it was confirmed that high concentration of HDTMA and gentamicin (200 mg/L) has a greater antibacterial activity than 50 mg/L of SK and GSK. The results did not only ensure the immobilisation of HDTMA and gentamicin on the surface of kaolinite, but also demonstrate the prominent use of local kaolinite as an active carrier for antibacterial agents and antibiotic compounds.

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