

Evaluation of phase transformation behaviors of zeolite and antibacterial properties against Gram-positive and -negative bacteria

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Characterization and Antibacterial Activity of Streptomycin Antibiotic Immobilized on Synthesized Zeolite from Natural Kaolinite

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Abstract

The escalating issues of environmental pollution coupled with growing antibiotic resistance as a result of bacteria release demands the development of efficient alternative antibacterial agents. To overcome this problem, antibacterial compounds could be loaded on a carrier system such as synthetic zeolite from kaolinite. Characterization results from X-ray diffraction, Fourier transform infrared spectroscopy, field emission scanning electron microscope with energy dispersive X-ray, transmission electron microscope and dispersion behaviour proved that a high purity of zeolite A was synthesized from kaolinite via alkaline hydrothermal method with high reproducibility. The zeolite A has been loaded with three concentrations of streptomycin (50, 100 and 200 mg/L). The cubic framework of zeolite A maintained after immobilization showing antibiotic molecules adsorption onto the zeolite surface. The antibacterial activity of the samples was determined using disk diffusion technique and minimum inhibition concentration against Gram-negative bacteria (*Escherichia coli* ATCC 11229) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538). Based on the antibacterial results, both types of bacteria were susceptible to the streptomycin loaded zeolite A with directly proportional to the streptomycin concentration. In conclusion, the synthesized zeolite A from natural kaolinite has a good prospect as a carrier system for streptomycin, generating a broad spectrum of antibacterial activity.

Keywords: zeolite, hydrothermal, kaolinite, antibacterial agent, streptomycin

1. Introduction

Increasing problem with antibacterial agents due to its high release into water necessitates the development of a carrier system for effective antibacterial agents. Multidrug resistance of pathogenic bacteria has become a significant threat not only to public health but also to food security and development. The problem is even more threatening when considering the slow paced development and limited number of new antimicrobial agents compared to the fast adaptation of the pathogens [1]. For example, *Enterococcus faecium* is not only resistance towards antibiotics but also to alcohol and other antibacterial compound in disinfectant solutions in hospitals such as hand rub sanitizer [2]. This is a worrying fact because alcohol based disinfectant solutions are vital to control infections worldwide.

Antibiotics are excellent example of antibacterial agent commercially used to facilitate the immune system to fight and stop the bacterial infections. Various antibiotics are available to treat infections and diseases nowadays. However, long term exposure to the environment and improper usage has developed antibiotic resistance bacteria [3]. Resistance happen because the bacteria evaluate the surrounding and adapts itself to overcome the antibiotics. Therefore, an improvement for antibacterial agent is needed to inhibit bacteria growth and infections which can lead to more serious diseases. Various techniques are used nowadays to develop new alternative effective antibacterial agent. One such example includes the immobilization of antibacterial compound onto a carrier system using zeolite [4].

Thus, this study aims to prepare and characterize synthesized zeolite from kaolinite which is abundant and cheap raw material in Malaysia [5]. Kaolinite is a good starting material for zeolite synthesis because its stable property minimize the impurity percentage of product by co-crystallization approach [6]. Additionally, the fact that kaolinite Si/Al ratio is almost 1:1 produces high purity zeolite. There are studies that showed successful synthesis of zeolite from kaolinite [7–9].

However, research on aminoglycoside loaded or immobilized on synthesized zeolite as antibacterial agent was scarcely reported. The efficiency of the combination was tested for their antibacterial activity against Gram positive and Gram negative bacteria. Hypothetically, streptomycin molecules are immobilized on the synthesized zeolite and will exert effective antibacterial activity. The presence of functional groups on streptomycin is responsible for its attachment on the zeolite which could be able to interact with the bacterial cell membrane and finally kill the bacteria.

This study fit to the expected finding where the effective antibacterial agent can be developed from functionalized zeolite with minimal release to the environment. With the combination of knowledge, the zeolite from kaolinite when loaded with aminoglycoside is expected to enhance the antibacterial properties and thus, solving related problems of antibiotics release into environment and bacterial resistant to antibiotics.

2. Experimental

2.1. Materials

Kaolinite (KM40) was purchased from Kaolin (M) Sdn. Bhd located at Tapah, Perak, Malaysia. The methodology used to synthesize zeolite was based on previous work [10] with some modifications on the crystallization time and temperature. During zeolite production, plastic based apparatus such as Teflon or Polytetrafluoroethylene (PTFE) bottles were used to prevent corrosion of glassware[11].

2.2. Synthesis of zeolite A from natural kaolinite

Kaolinite was converted to metakaolinite (MTK) by calcination at 900°C for 2 hours using a muffle furnace Carbolite model ELF 11/6B/301. Approximately 5.0 g of metakaolinite was mixed with 66.5 mL of NaOH solution using Teflon bottle and continuously stirred using magnetic stirrer for 2 hours in silicon oil at 70°C. After that, the mixture was left ageing for 20 hours at room temperature. After 20 hours, the mixture was let to crystallize at 100°C for 24 hours in a universal oven Memmert MMT-UN55. After taken out from the oven, the white mixture was filtered using Macherey-Nagel filter paper (125 mm) and rinsed a few times using warm distilled water. The solid portion was dried overnight in an oven at 80°C. The dried solid sample was ground using mortar and pestle, sieved into powder form and finally the powder sample was kept in a labelled clean container to be used for streptomycin adsorption.

2.3. Preparation of streptomycin loaded zeolite A

Streptomycin (STR) powder was purchased from Santa Cruz Biotechnology Inc. The adsorption of STR on the synthesized zeolite involved three different STR

concentrations which are 50, 100 and 200 mg/L. The STR stock solution was prepared by dissolving STR powder with distilled water according to the respective concentrations with slight modification [12]. About 0.5 g of the synthesized zeolite was added in 100 ml of STR solution in three beakers, respectively. Then, the mixture was stirred by using magnetic stirrer for 24 hours. After 24 hours stirring, the mixture was filtered using a filter paper to separate between the solid portions and the filtrate. The solid portion was dried overnight in an oven at 70°C. The recovered solid was ground and sieved into powder form for further usage. The plastic containers were labelled as 50ZS, 100ZS and 200ZS, respectively.

2.4. Characterization of the materials:

Approximately 2.0 g of powder samples were characterized by using X-ray diffraction (XRD) recorded on a Bruker D8 Advance diffractometer with Cu-K α radiation ($\lambda=1.5406 \text{ \AA}$, 40 kV, 20mA) within 2θ range of 10°-75° and scan step rate of 0.05°s⁻¹. Fourier transform infrared (FTIR) spectrophotometer Nicolet iS50 Thermo Scientific was used to investigate the functional groups of the samples using KBr pellet of ratio 1:5 or 1:10 (sample:KBr) equipped with OMNIC software. The detailed surface morphology and elemental analysis was observed using JEOL Model of field emission scanning electron microscope (FESEM) (JSM 6701-F) combined with energy dispersive X-ray (EDX) analyser software (analysisStation). Platinum coated sample was prepared due to its low electron conductivity thus producing best quality image. Transmission electron microscopy (TEM) analysis was used to analyse the 2D morphology and particle size of the synthesized zeolite powder using Hitachi HT7700 at 120 kV. Pre-treatment using ethanol was done to disperse the sample particles prior sonication and dropped onto carbon grid. Dispersion behaviour study was done to investigate the changes in physical properties of the zeolite (inorganic) when adsorbed with streptomycin (organic). Distilled water and n-hexane were used in this experiment. About 0.05 g of each sample was added into the mixture of 3 ml distilled water and 3 ml n-hexane in a clear glass bottle. The relative position of the samples in the mixture was compared using images at three conditions (1) immediately after the sample was added, (2) after mixture was shaken for 30 minutes at room temperature and (3) after 24 hours left in static condition.

2.5. Antibacterial assay

The antibacterial activity of all samples was evaluated against both Gram-positive and Gram-negative bacteria. Gram-positive bacteria used were *Staphylococcus aureus* (ATCC 6538) and Gram-negative bacteria *Escherichia coli* (ATCC 11229). For this assay, disk diffusion technique (DDT) and minimum inhibition concentration (MIC) were used to study their antibacterial activity for qualitative and quantitative analyses, respectively. Procedures were done strictly under aseptic condition to avoid contamination. Accurately, 0.2 g of each sample (ZEO, 50ZS, 100ZS, 200ZS) was pressed into pellet using hydraulic press at a pressure of 1400 psi using E-Z Press™ from International Crystal Laboratories for further use in DDT analysis. The Gram-positive bacteria; *S. aureus* (ATCC 6538) and Gram-negative bacteria; *E. coli* (ATCC 11229) were cultured on nutrient agar (NA) at 37°C for 24 hours. Approximately five to ten colonies of the cultured bacteria were diluted with sterile 0.9% saline solution and the suspension was adjusted equivalent to 0.5 McFarland standards (1.5×10^8 CFU (colony forming unit)). The sterile cotton swab was used to inoculate the surface of another NA plate by turning the plate every 60° to ensure homogenous bacteria growth. The sample pellet discs were placed on the surface of the agar plates gently at equal distances. The diameter of inhibition (in cm) was measured using a ruler after incubation of 24 hours at 37°C. MIC technique is an assay for testing the lowest concentration of antibacterial agent to completely inhibit bacterial growth. Briefly, 10% (v/v) of the bacteria culture was transferred in a 180 ml of fresh Luria-Bertani (LB) broth and was shaken at agitation rate of 200 rpm at 37°C until the optical density (OD) at $\lambda_{550\text{ nm}}$ reached in the range of 0.6-0.8. After that, the bacteria were transferred into falcon tubes and centrifuged at 4000 rpm for 15 minutes and the pellet was washed three times with sterile distilled water and 0.9% saline solution. Then, 10.0 mL of the prepared bacterial suspension in saline or distilled water was added into nine different ranges of sample concentrations (0.05, 0.1, 0.5, 1, 3, 6, 9, and 12 g/L). All samples were shaken for 30 minutes with agitation rate of 100 rpm at 37°C. After 30 minutes, 10 μL of bacteria solution was dropped onto MHA and the plate was incubated at 37°C for 16 hours. The plate was observed for the growth of the bacteria.

3. Results and discussion

3.1. Characterization

Figure 1(a) shows XRD diffractogram structural change of kaolinite (KAO), metakaolinite (MTK) and synthesized zeolite A (ZEO) which is a product of alkaline hydrothermal synthesis. The XRD pattern of the KAO displayed strong peaks at

12.34° and 24.64° that can be attributed to kaolinite [7]. Raw kaolinite has minor traces of quartz identified at 26.68° [13]. Quartz is one of a common impurities found in natural clays including the Malaysian clays due to weathering and natural decomposition of minerals [13]. Quartz could cause possible inflammation to skin if exposed at high concentration besides reduces crystal purity.

Metakaolinite is identified by the disappearance of all XRD peaks of the kaolinite replaced by background noise peaks indicating amorphous form. This happens due to decomposition of the kaolinite at high temperature of 900°C promoting structural change of kaolinite and thus, enhancing its reactivity for the synthesis of the zeolite [8].

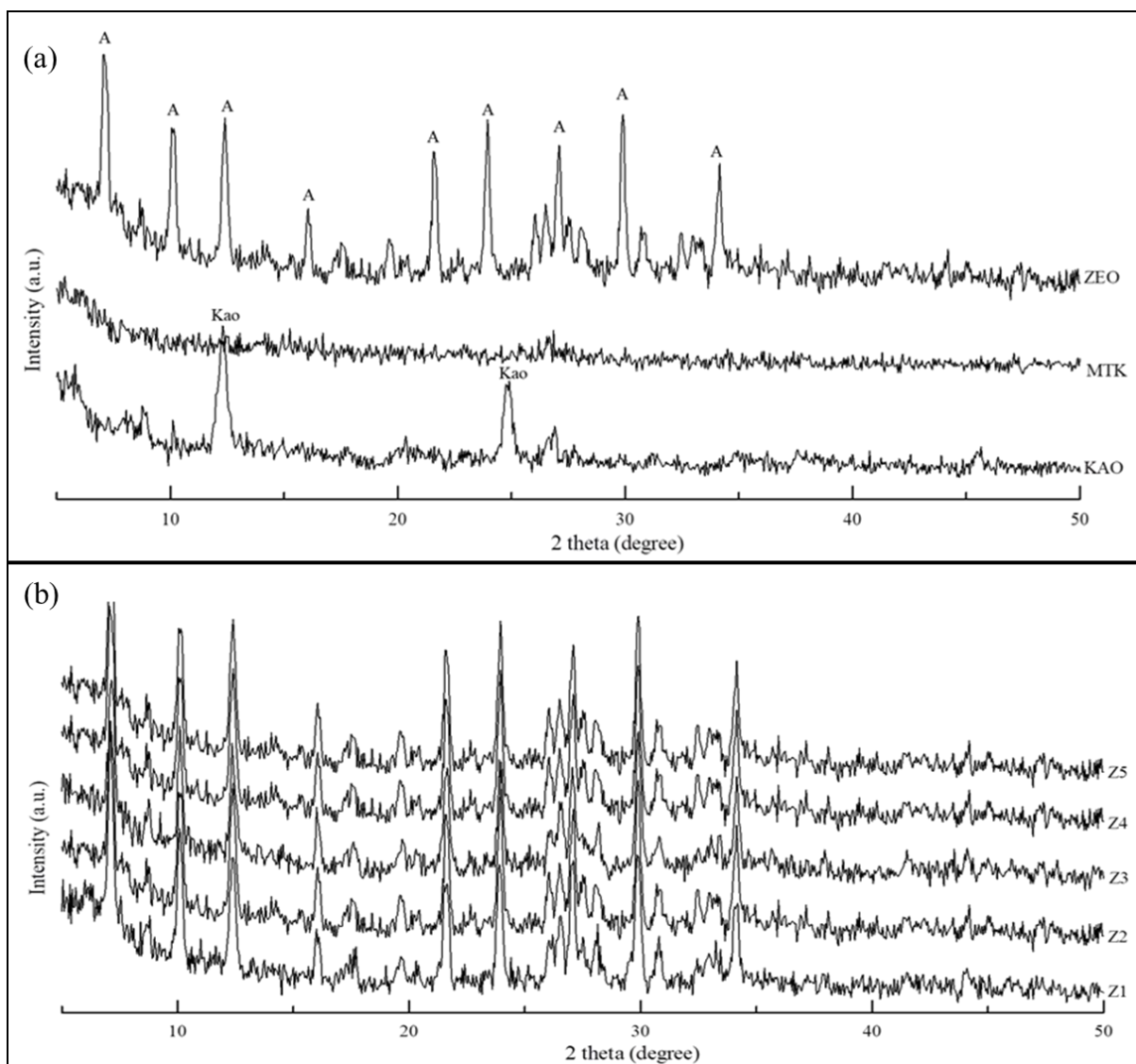


Figure 1 (a) XRD diffractogram of kaolinite (KAO), metakaolinite (MTK) and synthesized zeolite A (ZEO) with important peaks of zeolite A (Legend: A:

Zeolite A, Kao: Kaolinite) (b) XRD diffractogram of samples from different batches of synthesized zeolites (Z1-Z5)

The formation of synthesized zeolite A was confirmed by comparing the 9 significant peaks of the product obtained with the XRD pattern of sodium aluminium hydrate zeolite A [14]. The common peaks matched with that of zeolite A at 2θ of 7.13° , 10.12° , 12.40° , 16.2° , 21.63° , 23.94° , 27.08° , 29.90° and 34.14° . Elimination of quartz peak was successfully done by using this method thus producing high purity zeolite A. The XRD peaks of 5 batches of the synthesized from kaolinite using the same method as in Figure 1(b) showing high reproducibility of the zeolite A. This result indicates successful conversion of highly crystalline zeolite A from Malaysian local kaolinite mineral through alkaline hydrothermal technique with high reproducibility.

In the drug loading experiment, streptomycin molecules were loaded on zeolite A without the presence of new peaks found. Theoretically, zeolite type A has a rigid frame structure that is very close to one another so that it makes very low porosity making it harder for large ions to occupy inside the zeolite framework [15]. Thus, the adsorption activity is most probable occur at zeolite surface. The structure remains the same after modification because the cationic streptomycin drugs are adsorbed onto the zeolite surface and thus, will not affect the structure and framework of the zeolite. Successful adsorption of antibiotic molecules onto synthesized zeolite was accomplished while maintaining structural stability of the zeolite.

XRD analysis is strongly supported by results from FTIR spectroscopy. The chemical bonds and functional groups bonding involved verifying ZEO A conversion from raw KAO was shown in Figures 2 and 3. The transformation of kaolinite to zeolite A can be clearly observed from the FTIR spectra in the fingerprint region of $1100 - 450 \text{ cm}^{-1}$ and hydroxyl stretching region from 3000 to 3900 cm^{-1} .

Figure 2 shows important bonds related to the transformation of raw material to zeolite; (1) Si-O, (2) Al-OH and (3) OH. The raw kaolinite has well-defined spectra in this region due to Si-O, Si-O-Al, and Al-OH vibrations. After subjected to calcination at 900°C , most of the bonds in metakaolinite were disrupted indicating an amorphous state of material as shown in the FTIR spectrum of MTK. However, there is a small peak at 550 cm^{-1} , indicating the presence of Si-O-Si. Si-O-Si is a type of ionic bonding which is harder to be broken down compare to other bonds. This can be seen

clearly by the ZEO sample which indicates the disappearance of Al-OH peak at 910 cm^{-1} and the appearance of new peaks indicating strong Si-O bonds at 650 and 1000 cm^{-1} . The FTIR spectrum of the synthesized zeolite fits the peak characteristic of hydrated zeolite A. The peak at 1636 cm^{-1} was observed and attributed to water hydration. The presence of zeolitic material was confirmed through the peak of 1000 cm^{-1} which signifies abundant bonds of Si-O [16].

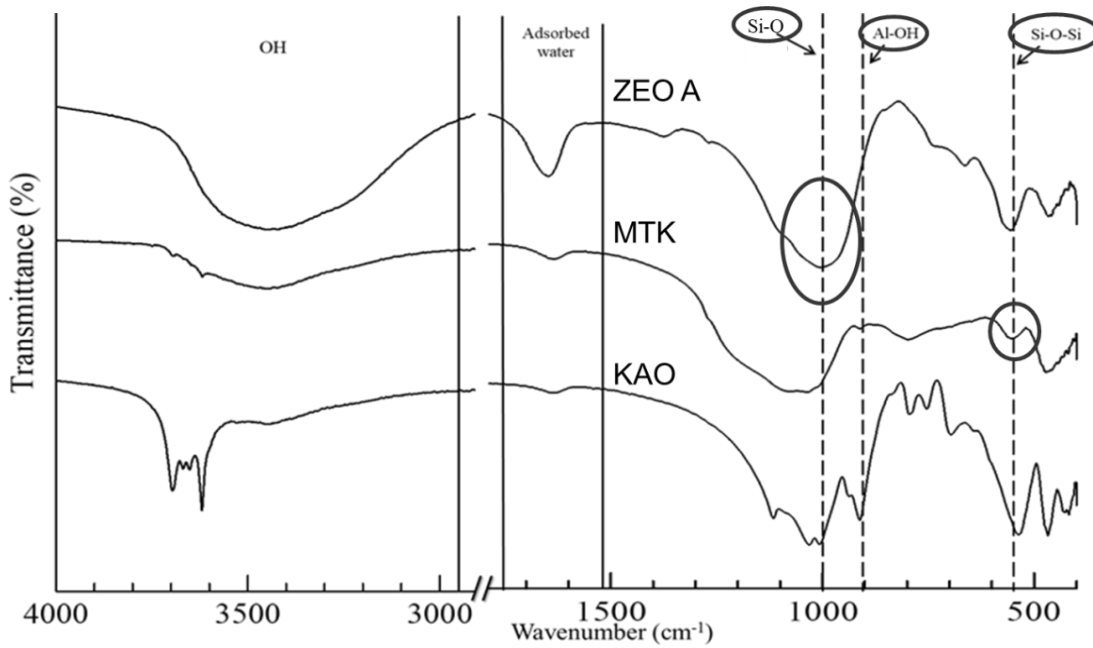


Figure 2 FTIR spectra of KAO, MTK and ZEO

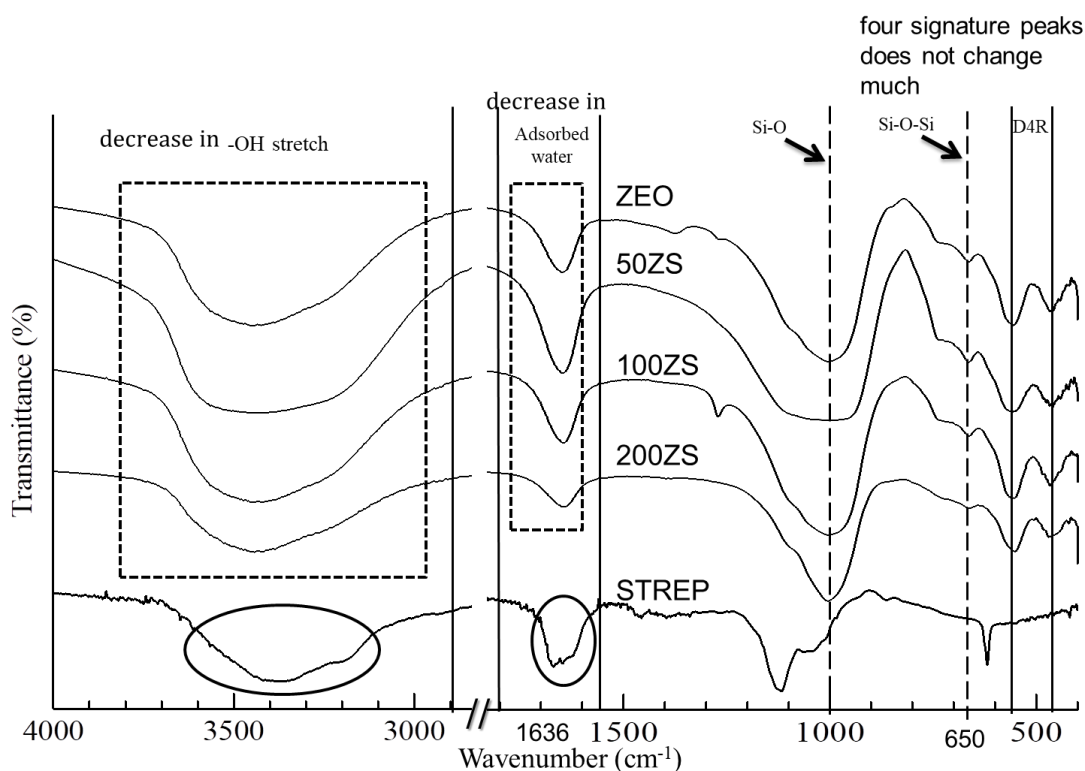


Figure 3 FTIR spectra of ZEO, 50ZS, 100ZS, 200ZS and streptomycin sulphate

All spectra in figure 3 shows consistent pattern of zeolite A with varying peak intensities in the fingerprint region between 1800 and 400 cm^{-1} and, hydroxyl stretching region from 3000 to 3800 cm^{-1} . The peaks around 650, 548 and 456 cm^{-1} indicate the zeolite A [17]. The peak at 548 cm^{-1} indicates the vibration of the four tetrahedral double ring (D4R), which is dominant in the secondary building unit of the zeolite A structure and the peak at 456 cm^{-1} represents internal vibration of D4R.

The presence of zeolite material was confirmed through the peaks below 1000 cm^{-1} which signifies abundant bonds of Si-O. These four signature peaks do not change much in terms of intensity and position of peaks for all samples and thus, conclude that the cubic structure of zeolite A is fairly maintained with all modified zeolite.

The peak at 1636 cm^{-1} was observed and attributed to the adsorbed water. As concentration of streptomycin loaded to zeolite increases (ZEO < 50ZS < 100ZS < 200ZS), there is a decrease in the intensity of hydroxyl stretching from 3000 to 3800 cm^{-1} and adsorbed water at peak 1636 cm^{-1} . These peaks are overlapped with the important -NH peaks of streptomycin which can be seen at 1650 cm^{-1} [18]. Figure 4 clearly proved that the loading of streptomycin onto synthesized zeolite A did not show any special peaks due to similar groups such as -OH and -NH with zeolite A.

This could be due to the stronger peak signal of hydrogen bonds and the inter-hydrogen bonds formed among the cationic antibiotic to the anionic zeolite A compared to control streptomycin solution. Thus, this resulting in the reduced intensity of the overlapped peaks and it is an indicator that the zeolite A material was successfully loaded with streptomycin molecules.

FESEM analysis was conducted to visualize the surface morphology of the samples. Figure 4 shows the morphology of the synthesized zeolite obtained from the kaolinite including streptomycin loaded zeolite (ZEO-STREP). Kaolinite can be observed by its platy morphology and thick book stack like structures [19]. After calcination, metakaolinite is shown as irregular amorphous material. The micrograph of ZEO shows a distinct character of zeolite A which has cubic crystal with sharp edges morphology after alkaline hydrothermal synthesis process. Figure 4 displays the presence of intergrowth twinning onto cubic shape of zeolite A with negligible low amount of amorphous and aggregate materials. Intergrowth twinning can be recognized by the presence of two cubic structure interpenetrate the face of zeolite crystal. This structural feature happens due to stacking fault at the sodalite cage at the early stage of nucleation [20]. Generally, the twin growth is a common observation similar to that found in fluorite crystal [21].

The crystals have an average particle size of 0.5 – 0.9 μm . Loiola *et al.* (2012) produced zeolite A having crystal size of 2-3 μm which are larger compared to this study. The difference might be due to the mixture that was subjected to ageing for 20 hours prior to crystallization stage at 100°C for 24 hours.

After that, the synthesized zeolite was loaded with antibiotic streptomycin sulphate. Interestingly, synthesized zeolite A did not change its cubic structure after adsorption with streptomycin as shown in Figure 4. In fact, the presence of antibiotic particles deposited on the zeolite surface can be observed clearly in Figure 4. The streptomycin particle is too large in size to enter the internal pores of zeolite A which are usually 0.3 – 1 nm and hence, they are adsorbed on the surface pores of the zeolites [22]. As an overall, significant morphology change from the starting material raw kaolinite until production of cubic zeolite A could be clearly observed. The synthesis process was found to be successful in producing a good degree of zeolite crystal with less impurity from raw kaolinite. Thus, FESEM results display the morphology of the cubic structure of zeolite A with particles of streptomycin on its surface proving the successful adsorption.

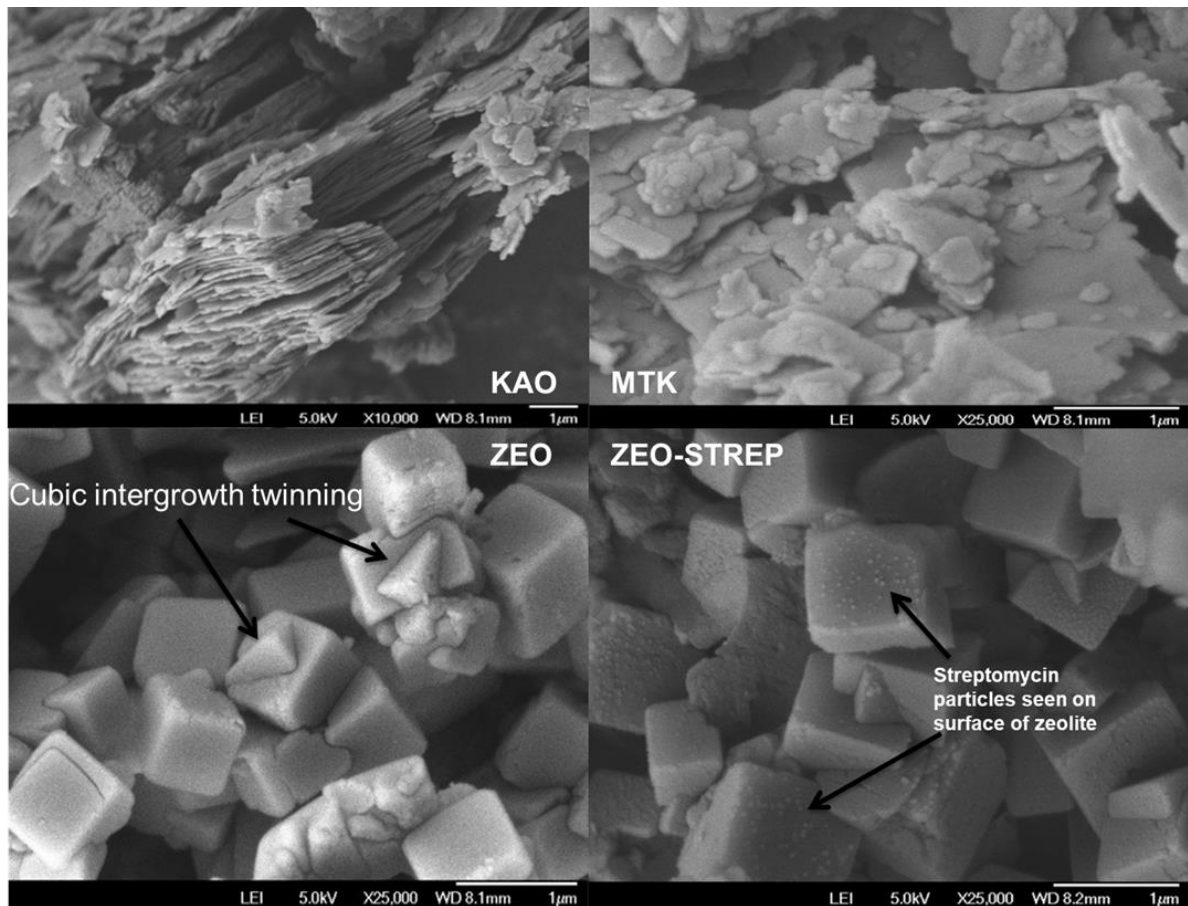


Figure 4 FESEM micrographs showing KAO, MTK ZEO and ZEO-STREP

TEM was used to study the crystal structure of the modified synthesized zeolite with streptomycin because it consists of identical structural unit called lattice. TEM only shows 2D images without image surface information which were best determined using FESEM. The regular patterns of the zeolite A are shown from the images. From SAED pattern in figure 5, regular single crystal is shown by the synthesized zeolite sample in $[1\ 0\ 0]$ and $[1\ 0\ 0]$ cubic formation. This confirmed that only zeolite A type was synthesized from kaolinite without any major impurities.



Figure 5 Selected area electron diffraction (SAED) pattern of ZEO-STREP



Figure 6 Dispersion behaviour of samples in hexane-water mixture

Finally, dispersion behaviour study (Figure 6) in an oil-water mixture showed that the streptomycin loaded zeolite become hydrophobic compared to all other material – kaolinite, zeolite and streptomycin. This analysis proved that the hydrophobicity can increase due to surface alteration of material. Hydrophobic

character of antibacterial agent will greatly attracts further disruptive interaction between bacterial membrane and cell wall components and thus, killing it.

The synthesis product of zeolite A from Malaysian local kaolinite mineral was successfully characterized using XRD, FTIR, FESEM, EDX, TEM and dispersion behaviour. The synthesized product has high crystallinity zeolite A, Si rich zeolite, smaller sized zeolite of 0.6 to 0.8 μm compared to previous method and established adsorption ability with antibiotic streptomycin via zeolite surface without any structural changes.

Meanwhile the FESEM micrographs show the expected morphology formation of the cubic structure zeolite A with visible particles of streptomycin on its surface. The TEM result highlights that the synthesized zeolite has single crystal composition of zeolite A and hence, strengthen the fact that the product has negligible impurities. The nature of streptomycin loaded zeolite A changed from hydrophilic to hydrophobic due to adsorption of antibiotic on its surface, making it favourable for antibacterial activity.

3.2. Antibacterial activity

The antibacterial assay of the samples was conducted against Gram-negative bacteria (*E. coli* ATCC 11229) and Gram-positive bacteria (*S. aureus* ATCC 6538) through disk diffusion technique (DDT) and minimum inhibition concentration (MIC). These two bacteria represent common found bacteria in wastewater and prone to cause infections to human [23].

Streptomycin loaded zeolite was prepared in three concentrations (50, 100 and 200 mg/L) and compared with control samples. Streptomycin solution disc (10 mg/L) was used as positive control while paper disc soaked with distilled water was used as negative control for each experimental set of *E. coli* and *S. aureus*.

Figure 7(a) and (b) shows the images of inhibition zone formed around the sample pellets of inoculated MHA surface containing bacteria. This result proved successful diffusion of streptomycin onto media containing bacteria. The inhibition zone for 200ZS disc is the biggest for both bacteria followed by 100ZS and finally, 50ZS. As the concentrations of streptomycin increases, the size of inhibition zone also increases and thus, showing increased effectiveness of antibacterial effect.

Furthermore, Table 1 shows that the inhibition zones for *S. aureus* are slightly bigger than *E. coli* for all streptomycin loaded zeolite samples. Streptomycin works

well on both bacteria but slightly higher inhibitory effect towards Gram positive bacteria. This might be due to the difference in cell wall composition where Gram positive bacteria has thick peptidoglycan layer whereas Gram negative bacteria possess a complex outer membrane layer which could act as a selective barrier for any antibacterial compound [24]. Hence, Gram positive bacteria is proven more susceptible towards streptomycin loaded synthesized zeolite compared to Gram negative bacteria.

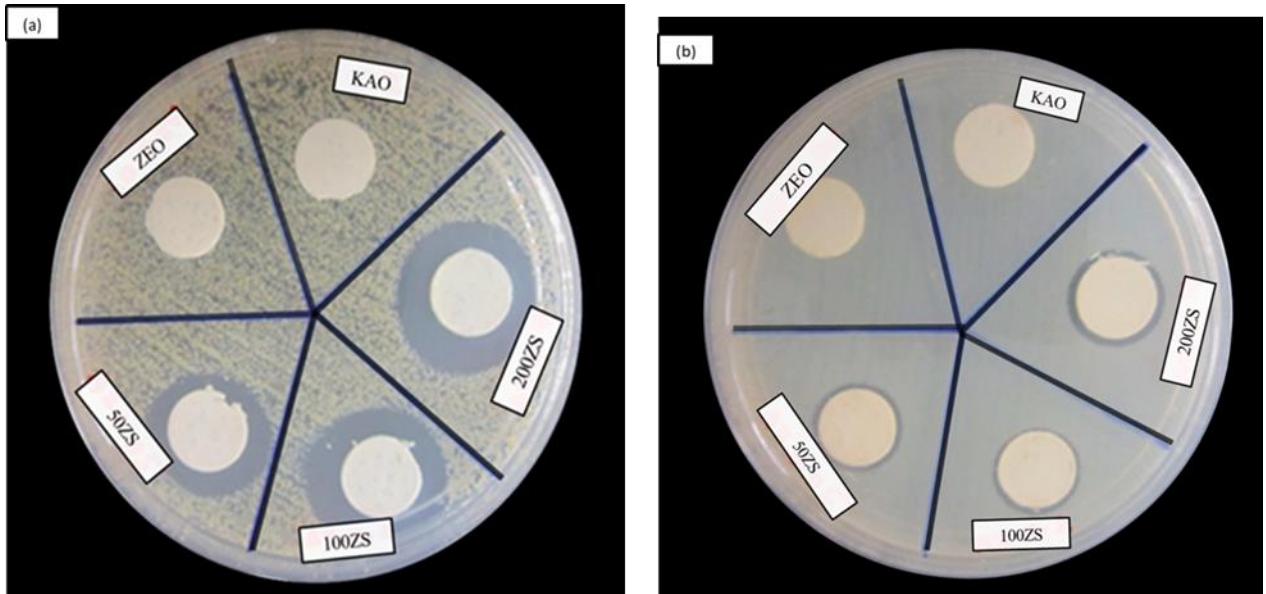


Figure 7 The images of the plate from DDT against (a) *S. aureus* ATCC 6538 and (b) *E. coli* ATCC 11229

Table 1: Inhibition zone diameter of the samples against bacteria

Samples	Inhibition zone (cm)	
	<i>E. coli</i> (Gram negative)	<i>S. aureus</i> (Gram positive)
STR	2.13	2.41
KAO	0.00	0.00
ZEO	0.00	0.00
50ZS	1.60	2.04
100ZS	1.87	2.20
200ZS	2.02	2.36

The antibacterial efficacy of zeolite loaded with streptomycin was probably due to the electrostatic adsorption of polycationic streptomycin to the negatively charged component of phospholipid membrane of bacteria as shown in Figure 7. This is followed by displacement of magnesium ions to enhance permeability and thus increase antibiotic uptake into cell [25]. Once into the cell, streptomycin molecule attacks the ribosome causing mistranslated proteins to form. Hence, this consequently leads to the cytoplasmic membrane damage that further facilitates subsequent streptomycin entry. The rapid uptake of additional aminoglycosides into the cytoplasm has caused an increased inhibition of protein synthesis and mistranslation and therefore accelerate cell death [25,26]. In other point of view, there could also be a hydrophobic attraction between functionalized zeolite and the lipophilic components of bacterial cell wall such as the phospholipids of Gram positive and the phospholipids and lipopolysaccharide (LPS) of Gram negative bacteria. The hydrophobic attraction also accelerates the action of streptomycin upon contact with bacteria [27]. The higher the concentration of streptomycin used, the bigger the inhibition zone due to the bacterial cell death.

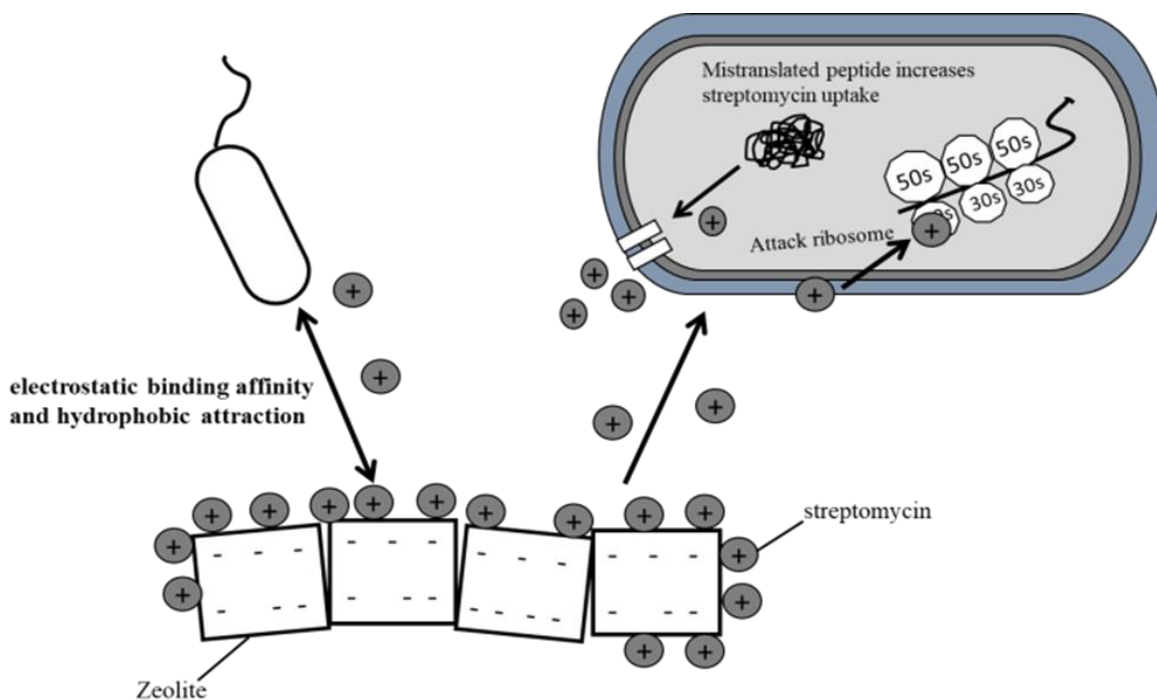


Figure 8 Possible mechanism of antibacterial action of streptomycin antibiotic immobilized on synthesized zeolite

MIC technique was used to determine the lowest concentration of antibacterial agent to completely inhibit bacterial growth from multiplying and reproducing visible growth. Streptomycin was used as a positive control in this study to show that the

antibiotic has high susceptibility towards both types of bacteria since it is a broad spectrum drug [28]. Both types of bacteria were susceptible to the modified zeolite with the lowest MIC value for Gram negative bacteria at 3.0 g/L in distilled water as shown in Table 1. Besides that, the antibacterial activity of streptomycin loaded zeolite is directly proportional to concentration of streptomycin used.

It is important to note that the antibacterial activity of streptomycin worked better in distilled water compared to saline water. This could be due to the nature of the streptomycin molecule which is strongly polar in water. Streptomycin is less efficient in saline environment due to chelating effect with Cl⁻ [29]. Salt medium is good in inhibiting bacterial growth causing the bacteria to lose water and die due to hypertonic environment [30]. Streptomycin works best at alkaline pH of about 8 to 9. Whereas, 0.9% saline solution for infusion has a pH around 5.5 which is acidic [31]. Therefore, there could be high chances that the pH change and thus, reducing the efficiency of antibacterial action.

Table 2: MIC values of samples against Gram positive bacteria and Gram negative bacteria in distilled water and 0.9% saline solution at 30 min incubation periods

Samples	MIC value (g/L) in 0.9% saline solution		MIC value (g/L) in distilled water	
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
STR	0.5	1	0.5	1
ZEO	>12	>12	>12	>12
50ZS	>12	>12	12	>12
100ZS	12	>12	6	9
200ZS	6	9	3	6

The streptomycin loaded synthesized zeolite A was proven effective in killing both Gram positive and Gram negative bacteria as stated from the DDT and MIC tests. The MIC value is the lowest for Gram negative bacteria (3 g/L) with better bactericidal effect in distilled water compared to saline environment. The major difference in cell wall composition of Gram positive and Gram negative bacteria contribute to the difference in their antibacterial activity. The higher the concentration

of streptomycin resulted in the higher effectiveness of antibacterial agent towards broad spectrum of bacteria.

4. Conclusion

Zeolite A was successfully synthesized from raw Malaysian kaolinite with high reproducibility. The synthesized zeolite was loaded with three concentrations of aminoglycoside streptomycin (50 mg/L, 100 mg/L and 200 mg/L) and then characterized using XRD, FTIR, FESEM, EDX, TEM and dispersion behaviour with prominent results. The zeolite A having 0.5 to 0.9 μm in size was synthesized with high crystallinity percentage and stable surface adsorption capacity towards streptomycin without any structural changes. This combination of synthesized zeolite and antibiotic is a good discovery since raw material is abundant in nature and hence, production is feasible and reproducible at low cost. From this study, it can be proposed that the antibacterial action of streptomycin towards bacteria comes from the hydrophobic and electrostatic affinity consequently defects the protein machinery. As a conclusion, this study provides more understanding of the antibacterial agent limiting its release into the environment.

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Highlights

- Zeolite A was synthesized from natural kaolinite via alkaline hydrothermal technique
- High purity and crystallinity, and lower particle size of synthesized zeolite A
- Smaller crystalline size of zeolite A enable high adsorption of streptomycin
- Positive antibacterial correlation with amount of streptomycin adsorbed on zeolite A