CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SYNTHESIZED ZEOLITE FROM NATURAL KAOLINITE LOADED WITH STREPTOMYCIN

ATIEYA BINTI ABDUL HADI

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DEDICATION

Allah, Rasulullah S.A.W and Islam

To my loving and dedicated mother,

Sharinahanim bt Rashid who taught me that even the largest task can be accomplished if it is done one step at a time with love and eagerness to learn

To my ever supporting and inspiring support system

Abdul Hadi bin Mahamood, Afieqah, Sabirah, Syifa' Auni

akhwati fillah

They taught me the value of grit and perseverance

because the best kind of knowledge is gained for the sake of Allah and helping this ummah.

And all who supported me during my research

Jazakumullahu khoiran kaseera

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ABSTRACT

The escalating problems of antibiotic resistance coupled with its growing environmental pollution as a result of rapid release of antibiotics demands the development of new and efficient antibacterial agents. To overcome this problem, antibacterial compounds are immobilized on a carrier system such as zeolite. In this study, zeolite A was successfully synthesized from natural kaolinite using alkaline hydrothermal method with sizes ranging from 0.5 to 0.9 µm. The product was used as a carrier system by loading it with three concentrations of streptomycin (50 mg/L, 100 mg/L and 200 mg/L). To characterize and validate the production of the zeolite A, the samples were characterized using X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, field emission scanning electron microscopy with energy dispersive X-ray (FESEM-EDX), transmission electron microscope (TEM) and dispersion behaviour. The XRD and FTIR results proved that zeolite A framework was maintained even after loading with streptomycin denoting its successful adsorption with minimal release. FESEM-EDX micrographs clearly indicated the presence of streptomycin particles on the cubic surface of zeolite A. In term of its dispersion behaviour, streptomycin-zeolite showed hydrophobic as compared to hydrophilic parent zeolite and thus, could attract bacterial cell wall. The antibacterial assay of the samples was conducted against Gram-negative bacteria (*Escherichia coli* ATCC 11229) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538) through disk diffusion technique (DDT) and minimum inhibition concentration (MIC). The DDT results indicated that the streptomycin released into the media because the inhibition zone values increased proportional to the increasing amount of streptomycin adsorbed onto zeolite A. 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. In conclusion, the synthesized zeolite A from raw kaolinite is a good adsorbent for antibiotic streptomycin, generating a broad spectrum antibacterial activity.

ABSTRAK

Peningkatan masalah ketahanan bakteria terhadap antibiotik serta pencemaran alam sekitar yang semakin menjadi-jadi rentetan pembuangan sisa antibiotik menuntut pembangunan agen antibakteria yang baru dan lebih efisien. Salah satu teknik yang boleh digunakan untuk mengatasi masalah ini adalah dengan menggabungkan sebatian antibakteria kepada suatu sistem pembawa seperti zeolit. Dalam kajian ini, zeolit A telah berjaya dihasilkan daripada kaolinit semulajadi menggunakan kaedah alkali hidrotermal. Produk ini digunakan sebagai ejen penjerap dengan pelbagai kepekatan streptomisin (50 mg/L, 100 mg/L and 200 mg/L). Untuk mencirikan hasil produk adalah zeolit A, sampel telah dicirikan menggunakan kaedah penyerakan sinar-X (XRD), spektroskopi infra-merah (FTIR), mikroskop imbasan pelepasan medan-serakan digabungkan dengan serakan tenaga sinar-X (FESEM-EDX), mikroskop penghantaran elektron (TEM) dan sifat penyebaran. Keputusan dari XRD dan FTIR membuktikan bahawa struktur zeolit A tidak terjejas selepas penjerapan streptomisin. Mikrograf FESEM-EDX menunjukkan kehadiran zarah streptomisin pada permukaan kubik zeolit A. Di samping itu, hasil analisis sifat penyebaran menunjukkan bahawa penjerapan streptomisin-zeolit meningkatkan sifat hidrofobik dan menggalakkan interaksi dengan dinding sel bakteria. Ujian antibakteria telah dilakukan terhadap bakteria Gram-negatif (*Escherichia coli* ATCC 11229) dan bakteria Gram-positif (*Staphylococcus aureus* ATCC 6538) melalui teknik serakan cakera (DDT) dan kepekatan perencatan minimum (MIC). Keputusan DDT menunjukkan bahawa streptomisin dilepaskan sedikit demi sedikit ke dalam media dan saiz zon perencatan meningkat secara berkadar langsung dengan kepekatan streptomisin pada zeolit A. Kedua-dua jenis bakteria telah dihalang pertumbuhannya dengan nilai perencatan minima serendah 3.0 g/L terhadap bakteria Gram negatif dalam air suling. Kesimpulannya, zeolit yang diproses dari kaolinit semulajadi merupakan ejen penjerapan yang baik untuk streptomisin serta mempunyai spektrum antibakteria yang luas.

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LIST OF ABBREVIATIONS

ATCC	-	American Type Culture Collection
Al	-	Aluminium
BD	-	Bacto Difco
CEC	-	Cation Exchange Capacity
CFU	-	Colony Forming Unit
DDT	-	Disk Diffusion Technique
EDX	-	Energy Dispersive X-ray
FESEM	-	Field Emission Scanning Electron Microscope
FTIR	-	Fourier Transform Infrared
IUPAC	-	International Union of Pure and Applied Chemistry
LB	-	Luria-Bertani
MHA	-	Muller Hinton Agar
MIC	-	Minimum Inhibition Concentration
mRNA	-	Messenger Ribonucleic Acid
MRSA	-	Methicillin-Resistant *Staphylococcus aureus*
NA	-	Nutrient Agar
OD	-	Optical Density
OH	-	Hydroxyl
PTFE	-	Polytetrafluoroethylene
rpm	-	Revolutions per minute
rRNA	-	Ribosomal Ribonucleic Acid
S	-	Subunit
STR	-	Streptomycin
TEM	-	Transmission Electron Microscope
tRNA	-	Transfer Ribonucleic Acid

LIST OF SYMBOLS

%	-	Percentage
µm	-	Micrometer
Å	-	Angstrom
cm	-	Centimeter
cm^{-1}	-	Reciprocal Centimeter
g	-	gram
kV	-	Kilovolt
M	-	Molar
mA	-	Milliampere
mg/L	-	Milligram per Liter
mol/L	-	Mol per Litre
nm	-	nanometer
º	-	Degree
ºC	-	Degree Celcius
Psi	-	Pounds per Square Inch
v/v	-	Volume per Volume
w/v	-	Weight per Volume
θ	-	Theta
λ	-	Lambda
µL	-	Microliter

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CHAPTER 1

INTRODUCTION

1.1 Problem Background

Most of the existing bacteria in environment are actually harmless to people. However, the presence of pathogenic bacteria that passes resistant plasmid among bacteria has gradually threatening human health (Salyers and Whitt, 2002). This problem arose from the water pollution of antibiotics detected from hospital effluent, domestic water and wastewater treatment plant effluent due to improper handling and its extensive use (Proia *et al.*, 2016). As a result, the balance of the bacterial communities was disrupted. One such example that showed antibiotic resistance of the bacteria include towards aminoglycoside due to its wide prescription among children and neonatal units at the hospital (Germovsek, Barker and Sharland, 2017). The emergence of multidrug resistant bacteria in the world nowadays urges the development of new alternative effective antibacterial agent.

One effective way is by immobilizing the antibacterial agents with the zeolite carrier system (Franci *et al.*, 2015). Zeolite is a type of aluminosilicate carrier that has a capability of adsorbing and retaining certain valuable compounds. This is due to its unique properties such as large cation exchange capacity (CEC), stable in harsh environment, molecular sieve property, high surface area and porosity (Lutz, 2014). Zeolite comprises of natural and synthetic zeolites. Both synthetic and natural zeolites exhibit strong affinity for water thus act as excellent adsorbent agent (Ghasemi and Younesi, 2011). They are widely been used for many applications such as catalysts, ion exchangers and adsorbents in the oil and gas industry (Azizi, Dehnavi and Joorabdoozha, 2013). Synthetic zeolite has advantages over the natural zeolite depending on its usage. However, the natural zeolite is not highly pure and it contains high amount of other impurities such as heavy metals and silica which are hazardous to human (Rios, Williams and Maple, 2007). Thus, the synthetic zeolites which can be synthesized from various raw materials producing highly pure zeolite and it can be tuned based on specification and is a suitable material for antibacterial agent especially for human purpose (Franus, Wdowin and Franus, 2014).

Aminoglycosides are antibiotics that are strongly cationic at physiological pH. These broad spectrum drug comprises of streptomycin, kanamycin, tobramycin and gentamicin which are used worldwide due to their high efficacy and low cost (Germovsek, Barker and Sharland, 2017). Aminoglycoside works effectively by targeting the ribosomes and thus producing defective protein (Ezraty *et al.*, 2013). Since zeolite has a negative charge on its surface as well as in its framework, therefore the cationic aminoglycoside molecules are suitable in the modification of zeolite surfaces. The function of antibiotics as preventive drug and treatment of infection can be improved. Recent studies showed the synergistic properties of nanoparticles with antibiotics for enhancement of their bactericidal properties (Nasirmahaleh *et al.*, 2016).

Therefore, this study focusing on developing new alternative for effective antibacterial agent derived from functionalized synthetic zeolite synthesized from local natural kaolinite. Some types of clays that can be found abundantly in Malaysia are common clay, ball clay, bentonite, fire clay, and kaolinite. According to the Malaysian Minerals Yearbook 2013, Malaysia has abundance of clay resources estimated at 685 metric tons (Mt) (Pui-Kwan Tse, 2013) and there are huge availability of kaolinite in peninsular Malaysia. The abundance of clay deposited in Malaysia is mainly exploited for the production of local tile, brick, ceramic and plastic. The local natural kaolinite mineral from Malaysia can be used as a cheap raw material in the synthesis of highly pure and valuable synthetic zeolites.

Previous findings have shown successfully synthesized several synthetic zeolites from kaolinite based on hydrothermal transformation of kaolinite and metakaolinite (Holešová *et al.*, 2014). This porous material is proven to have high chemical stability, thermal resistance, safe to user and long lasting action period (Johnson and Arshad, 2014). The synthesized zeolite from kaolinite has been functionalized using several functional groups such as (3-aminopropyl) triethoxysilane APTES (Hanim, Malek and Ibrahim, 2016) and quaternary ammonium compound (Malek and Ramli, 2015). There are also many studies of immobilization of antibacterial agent on zeolite such as silver ions on functionalized zeolite Y (Hanim *et al.*, 2016), copper ion exchanged zeolite Y (Ahmad *et al.*, 2018) and amoxicillin on surfactant modified zeolite (Nasirmahaleh *et al.*, 2016). The latest study showed that copper exchanged zeolite Y from rice husk ash has antibacterial activity against Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria (Ahmad *et al.*, 2018). To date, there is less study done on synthesized zeolite from Malaysian kaolinite and used as a carrier system for antibiotics.

1.2 Problem Statement

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 in 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 (WHO, 2017). 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 (Pidot *et al.*, 2018). 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. A variety of antibiotics are available to treat infections and diseases nowadays. However, long term exposure to the environment and improper usage has developed antibiotic resistance bacteria. Resistance happen because the bacteria evaluates the surrounding and adapts itself to overcome the antibiotics (Rodríguez-Rojas *et al.*, 2013). 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 (Franci *et al.*, 2015).

Thus, this study aims to prepare and characterize synthesized zeolite from kaolinite which is abundant and cheap raw material in Malaysia. There are studies that showed successful synthesis of zeolite from kaolinite (Ríos, Williams and Castellanos, 2010; Johnson and Arshad, 2014; Abdullahi, Harun and Othman, 2017). However, research on aminoglycoside loaded 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 is loaded on the synthesized zeolite and will exert effective antibacterial activity. The presence of functional group of streptomycin attached on the zeolite is 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 thus solving related problems.

1.3 Research Objectives

(a) To synthesize and characterize zeolite A from natural kaolinite as a drug carrier system

(b) To load streptomycin on synthesized zeolite with different streptomycin concentations and characterize them

(c) To study the antibacterial efficacy of streptomycin loaded synthesized zeolite and compare with the synthesized zeolite without drug

1.4 Scope of the study

This study involved the hydrothermal synthesis and characterization of zeolite from raw kaolinite (KM40) as an aluminosilicate source. The streptomycin loaded synthesized zeolites were prepared by adsorbing streptomycin solution at different range of concentrations (50 mg/L, 100 mg/L and 200 mg/L). Raw kaolinite (KAO), metakaolinite (MTK), synthesized zeolite (ZEO), 50ZS, 100ZS and 200ZS were characterized by using Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), field emission scanning electron microscope (FESEM) and also dispersion behaviour. The antibacterial activity was then investigated using disk diffusion technique (DDT) and minimum inhibition concentration (MIC) against Gram-positive bacteria; *S. aureus* (ATCC 6538) and Gram-negative bacteria; *E. coli* (ATCC 11229). The overall framework of this study is shown in Figure 1.1.

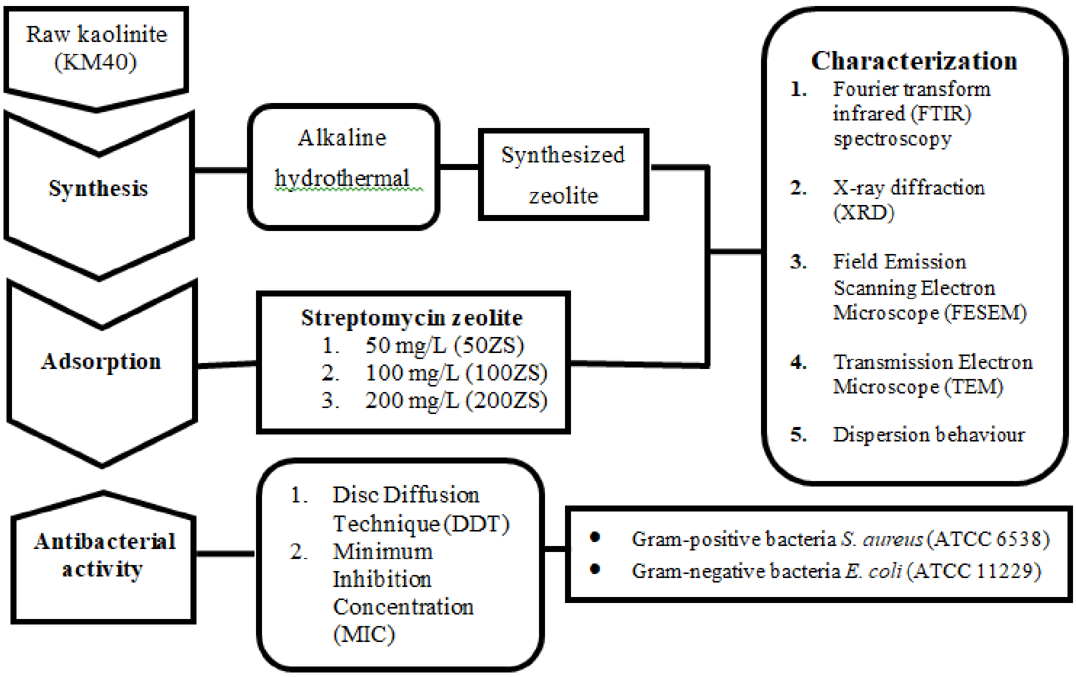

Figure 1.1 Framework of the overall study

1.5 Significance of Study

This study has been performed towards solving problems with antibacterial agents mainly the bacterial resistance and also environmental pollution caused by the release of the antibacterial agents into the environment. Bacterial resistance to antibacterial agents occur because the bacteria defend themselves from being killed by the antibacterial agent. Each attack from the bactericide triggers the bacteria to record, identify and change the permeability of its outer membrane as a defence mechanism. Resistance is the result of the adaptation done by the bacteria to prevent antibiotic from entering its cell membrane (Delcour, 2010). The emergence of antibiotic resistance increases the number of unknown symptoms or advance diseases, making the treatment more expensive and tedious. This study could provide more understanding of the antibiotic action in killing the bacteria. Thus the problem of antibacterial resistance can only be cure by advancing the development of effective antibacterial agent and besides reducing environmental pollution.

In fact, the zeolite carrier system was synthesized using kaolinite as a raw material. As mentioned previously, the kaolinite is abundant and readily available at low price in Malaysia. Therefore the cost of the production can be reduced. This combination of synthesized zeolite and antibiotic will pave way for more research since the production is feasible, able to be produced in large amount and save cost. The functionalized zeolite is also expected to kill broad spectrum of bacteria according to the type of antibiotic loaded to it. Besides, the end product is safe to the environment and have potential to act as adsorbent when released into environment.

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