CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF HEXADECYLTRIMETHYLAMMONIUM MODIFIED SILVER KAOLINITE

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Specially dedicated to:

My inspring grandparents;

My grandfather, M. Komarasami

My grandmother, R. Thanapakiam

My loving parents;

My father, K. Muthoovaloo

My mother, J. Susila

My beloved fiancée;

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ABSTRACT

The current study focused on the preparation, characterization and evaluation for antibacterial activity of hexadecyltrimethylammonium modified silver kaolinite (DUAL-ANTIBAX®). DUAL-ANTIBAX® was prepared by adding Ag-Kaolinite with surfactant, hexadecyltrimethylammonium (HDTMA). The prepared samples; Kao (raw kaolinite), Ag-Kao, HDTMA-Kao and DUAL- ANTIBAX® were characterized using an X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, field emission scanning-electron microscopy (FESEM), energy dispersive X-ray (EDX) analyser, zeta potential analysis and dispersion behaviour. The samples were observed to have no structural changes and HDTMA and Ag were successfully loaded on kaolinite. DUAL-ANTIBAX® has a positive value of zeta potential due to the presence of surfactants. The antibacterial activity of these samples was determined by Minimum Inhibition Concentration (MIC) assay against *Staphylococcus aureus* and *Escherichia coli* in different saline solution concentrations (0%, 0.01%, 0.1%, 1.0% and 5.0%) and at different time of incubation (30 minutes and 24 hours). As a result, DUAL-ANTIBAX® showed better antibacterial activity at lower concentration of saline solution. Meanwhile, DUAL-ANTIBAX® exhibited better antibacterial activity at time of incubation of 24 hours compared to 30 minutes. This study also revealed that, DUAL-ANTIBAX® was more effective on the Gram positive bacteria compared to the Gram negative bacteria. As a conclusion, DUAL-ANTIBAX® can be used as an effective antibacterial agent due to the combination of Ag and HDTMA on kaolinite as a carrier system. Results from this study has highlighted the potential use of DUAL-ANTIBAX® as antibacterial agent and may have an implications to the development of new antibacterial agent.

ABSTRAK

Kajian ini bertujuan untuk menyediakan, mencirikan dan menilai aktiviti antibakteria heksadesiltrimetil ammonium diubahsuai dengan perak kaolinit (DUAL-ANTIBAX®). DUAL-ANTIBAX® telah disediakan dengan mengubahsuai ion perak dan surfaktan, heksadesiltrimetil ammonium (HDTMA) pada kaolinit. Kajian pencirian telah dijalankan terhadap sampel kaolinit (Kao), perak-kaolinit (Ag-Kao), surfaktan-kaolinit (HDTMA-Kao) dan DUAL-ANTIBAX® dengan menggunakan kaedah pembelauan sinar-X (XRD), spektroskopi inframerah (FTIR), mikroskopi imbasan electron (FESEM), penganalisis tenaga serakan sinar-X (EDX), analisis potensi zeta dan sifat-sifat penyebaran. Kesemua sampel didapati tidak mengalami sebarang perubahan struktur. Manakala HDTMA dan Ag didapati telah berjaya dimuatkan pada kaolinit. Disebabkan kehadiran surfaktan, DUAL-ANTIBAX® mempunyai nilai positif bagi analisa zeta potensi. Kemudian, aktiviti antibakteria telah ditentukan dengan menggunakan kaedah asai kepekatan perencatan minimum (MIC) terhadap *Staphylococcus aureus* dan *Escherichia coli* dalam kepekatan larutan garam yang berbeza (0%, 0.01%, 0.1%, 1.0% dan 5.0%) dan tempoh masa inkubasi yang berbeza (30 minit dan 24 jam). Berdasarkan nilai MIC, DUAL-ANTIBAX® menunjukkan aktiviti antibakteria yang lebih baik pada kepekatan garam yang rendah. DUAL-ANTIBAX® juga mempunyai aktiviti antibakteria yang lebih baik pada masa inkubasi 24 jam berbanding dengan 30 minit. Di samping itu, DUAL-ANTIBAX® didapati lebih berkesan terhadap bakteria Gram positif berbanding bakteria Gram negatif. Kesimpulannya, DUAL-ANTIBAX® boleh digunakan sebagai agen anti-bakteria yang berkesan disebabkan gabungan Ag dan HDTMA pada kaolinit sebagai sistem pembawa. Keputusan kajian ini telah menekankan potensi penggunaan DUAL-ANTIBAX® sebagai agen antibakteria dan mungkin mempunyai implikasi kepada pembangunan agen antibakteria baru.

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LIST OF ABBREVIATION

AgCl	-	Silver Chloride
$AgNO_3$	-	Silver Nitrate
AgNP	-	Silver nanoparticles
Ag-Kao	-	Silver-Kaolinite
BKC	-	Benzalkonium Chloride
CEC	-	Cation Exchange Capacity
CPB	-	Cetylpyridinium Bromide
Cl^-	-	Chloride
Cha	-	Chabazite
Cli	-	Clinoptilolite
CPC	-	Cetylpyridinium chloride
CTAB	-	Cetyltrimethyl Ammonium Bromide
Cu	-	Copper
EDX	-	Energy Dispersive X-ray
FESEM	-	Field Emission Scanning-Electron Microscopy
FTIR	-	Fourier Transform-Infrared Spectroscopy
HDTMA	-	Hexadecyltrimethyl Ammonium
HDTMA-Kao	-	Hexadecyltrimethyl Ammonium-Kaolinite
Kao	-	Kaolinite
LB	-	Luria Bertani

MIC	-	Minimum Inhibitory Concentration
MMT	-	Montmorillonite
NA	-	Nutrient Agar
NaCl	-	Sodium Chloride
OD	-	Optical Density
QAC	-	Quartenary Ammonium Compound
SSD	-	Silver sulfadiazine
XRD	-	X-Ray Diffraction
Zn	-	Zinc

LIST OF SYMBOL

ºC	-	Degree Celsius
cm	-	Centi meter
g	-	Gram
g/l	-	gram/liter
kV	-	Kilo Volt
L	-	Liter
min	-	minute
ml	-	Milliliter
mM	-	Milli-molar
nm	-	Nanometer
ppm	-	Parts per million
rpm	-	round per minute
w/v	-	weight/volume
µL	-	Micro Liter
µm	-	Micro meter
À	-	Angstrom
λ	-	Lambda
θ	-	Theta

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CHAPTER 1

INTRODUCTION

1.1 Introduction

In the 20th century, the discovery and development of antibiotics are well known for most significant medical successes which are aimed to reduce illness and kill harmful bacteria (Otto and Haydel, 2013). Antibiotics may work in several ways such as inhibiting bacterial cell wall synthesis, protein synthesis or DNA synthesis. Consequently, this stops the bacteria from multiplying (Alalem, 2008).

However, the overuse and misuse of antibiotics proved to have an adverse effects on human body and may increase the number of infections which are caused by antibiotic resistance bacteria (Otto and Haydel, 2013; Sasidharan *et al.*, 2008). According to World Health Report (2007), antibiotic resistance has contributed to the emergence and re-emergence of infectious diseases (Syed *et al.*, 2010). This phenomenon has led to increase in morbidity, mortality and health care cost. These risks are expected to continue unless the problem of antibiotic resistance can be solved (Eliopoulos *et al.*, 2003).

Non-healing wound is a significant problem in health care system as millions of people are suffering from burns, injury, surgery and illness. Patient with thermal

injury requires immediate care because the wound can be easily infected by bacteria rapidly (Syed *et al.*, 2010). Once wound becomes infected, the healing will be delayed and scarring will occur due to toxic substances released by the pathogens (Atiyeh *et al.*, 2007; Sasidharan *et al.*, 2008). Infections usually require proper wound care time, expensive drugs and antibacterial therapies and may increase morbidity (Gunasekaran *et al.*, 2011). These socioeconomic impacts on wound management certainly emphasize the need for acceleration of wound healing process, reduce scarring and improve appearance of the healed wound.

In view to this, wound healing devices of present were used to simultaneously deliver number of active agents to wound site. One of the strategies that gain renewed attention is an invention of wound healing agents with antibacterial properties. This current study focusing on the characterization and antibacterial activity of DUAL-ANTIBAX® as potential wound healing agent with antibacterial activity.

Antibacterial materials can be divided into organic group and inorganic group. Organic antibacterial agents have few shortcomings such as low resistance to processing conditions which limits their applications (Tang and Lv, 2014). As a result, inorganic antibacterial agents have drew much interest in bacterial control.

Therefore, DUAL-ANTIBAX® has been proposed as a better antibacterial agent through this research. Two antibacterial agents, metal ion and quaternary ammonium compound (QAC) are attached on kaolinite (Malek and Isti'anah, 2013). This clay based antibacterial agent has been proven to be very effective against a wide range of microorganism. Since the compound consists of two antibacterial agents, it was expected to inhibit bacterial infection and at the same time decrease toxicity which caused by metal ions such as silver.

Metal ion such as Ag ions are attached on the surface of kaolinite clay by cation exchange in the mixture of 40-60% of their composition in order to produce

metal-clay. After that, quaternary ammonium compound such as HDTMA is added to the metal clay in order to produce DUAL-ANTIBAX® (Malek & Isti'anah, 2013). Metal ions are attached inside the framework of the clay, meanwhile QAC molecules are attached on the outer surface of the clay framework since QAC molecules are larger than metal ions. The ratio of both QAC and metal ions should be equal so that both agents able to kill wide spectrum of bacteria effectively (Malek and Isti'anah, 2013).

1.2 Problem Statement

Currently increasing number of antibiotic resistance bacteria lead to many complication such as higher treatment cost, longer hospital stay, infections and deaths (Eliopoulos *et al.*, 2003). Many researches have been going on to find alternative solutions and usage of antibacterial agents is encouraged. Silver has been known to possess antimicrobial effects with properties of conductivity, stability and activity (Afiqah and Rahim, 2012). Thus, one of the alternative way is using silver based product as antibacterial agent due to wide spectrum of antibacterial activity (Malek *et al.*, 2013). Silver is capable of killing the microorganisms on external wound in living tissues. This is the reason of physicians use silver sulfadiazine and silver nanoparticles as wound dressings to treat external infections (Percival *et al.*, 2005).

Many bacteria infect the wound on the surface, where they colonize and form complex communities which known as biofilm. They are notoriously resistant to antibiotics. Some of the antibacterial agents failed to kill or inhibits the growth of pathogens (Zhou, 2011). Besides that, prolonged or incomplete wound healing process has been a major problem people facing worldwide. Various efforts are being made to discover natural agents that can expedite healing process and at the same time reduce other complications. Therefore, the demand for cheap, easy to handle and promising antimicrobial agents keep on increasing.

With the alarming situation of microbial resistance against several antibiotics, development of antimicrobial coatings such as silver or quaternary ammonium cations which prevent bacterial attachment and biofilm formation has become a very promising research in wound care in recent years (Knetsch and Koole, 2011). Besides that, current market lacks of a long term application that would enable healing therapy without using a systemic treatment. In order to overcome this issue, a suitable carrier that can transport the substance to targeted place. DUAL-ANTIBAX® can be considered as one of the prominent agent which will be able to kill a wide spectrum of phatogenic microorganisms (Malek *et al.*, 2013).

In this research, silver ions were intercalated on a kaolinite clay as a carrier system. The effectiveness of these materials was determined by Minimum Inhibitory Concentration (MIC) test. The MIC was tested on different saline solution concentration and different time of incubation. Saline solution contains chloride ions (Cl^-) which carries negative charge while silver ions carry positive charge. Therefore electrostatic force will established in between these two charges which consequently form precipitated AgCl. However, this precipitate could make silver ions lose its antibacterial properties. Thus, silver ions will not be able to inhibit growth of bacteria (Atiyeh *et al.*, 2007). Moreover, wound exudate contains of anions which able to effect antibacterial activity of silver (Cutting, 2003). To overcome this, the presence of surfactants such as hexadecyltrimethylammonium (HDTMA) compounds are required since QAC has affinity towards negatively charged ions (Ullah *et al.*, 2014). tissues. This is the reason of physicians use silver sulfadiazine and silver nanoparticles as wound dressings to treat external infections (Percival *et al.*, 2005).

1.3 Research Objectives

This study focused on studying the antibacterial activity of DUAL-ANTIBAX®. The objectives of the study were as follows:

i) To prepare and characterize HDTMA modified silver kaolinite (DUAL-ANTIBAX®)
ii) To study the effect of different saline concentration and time period on the antibacterial activity of DUAL-ANTIBAX®

1.4 Scope of Research

This research was divided into 2 stages whereby each stage was designed to achieve the objectives of the research. First part of the research methodology was aimed to prepare and characterize the studied samples. In the stage 2, antibacterial activity of the studied samples against *E. coli* and *S. aureus* was determined by Minimum Inhibition Concentration (MIC) assay. Figure 1.1 and Figure 1.2 illustrate general overview of the research.

At the first stage of the study, samples namely kao (raw kaolinite), Ag-Kao (silver-kaolinite), HDTMA-Kao (surfactant modified kaolinite) and DUAL-ANTIBAX® (surfactant-silver-kaolinite) were prepared and characterized. The characterization of studied samples was carried out to analyse their structural, elemental and morphology of the samples using X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, field emission scanning-electron microscopy (FESEM), energy dispersive x-ray (EDX), zeta potential analysis and dispersion behaviour. At the next stage, the antibacterial activity of studied samples

was evaluated using Minimum Inhibitory Concentration (MIC) assay in different percentage of saline concentration (0%, 0.01 %, 0.1 %, 1.0 % and 5.0 %) and two different times of incubation (30 min and 24 hours). *E. coli* and *S. aureus* have been used as model microorganisms in this study.

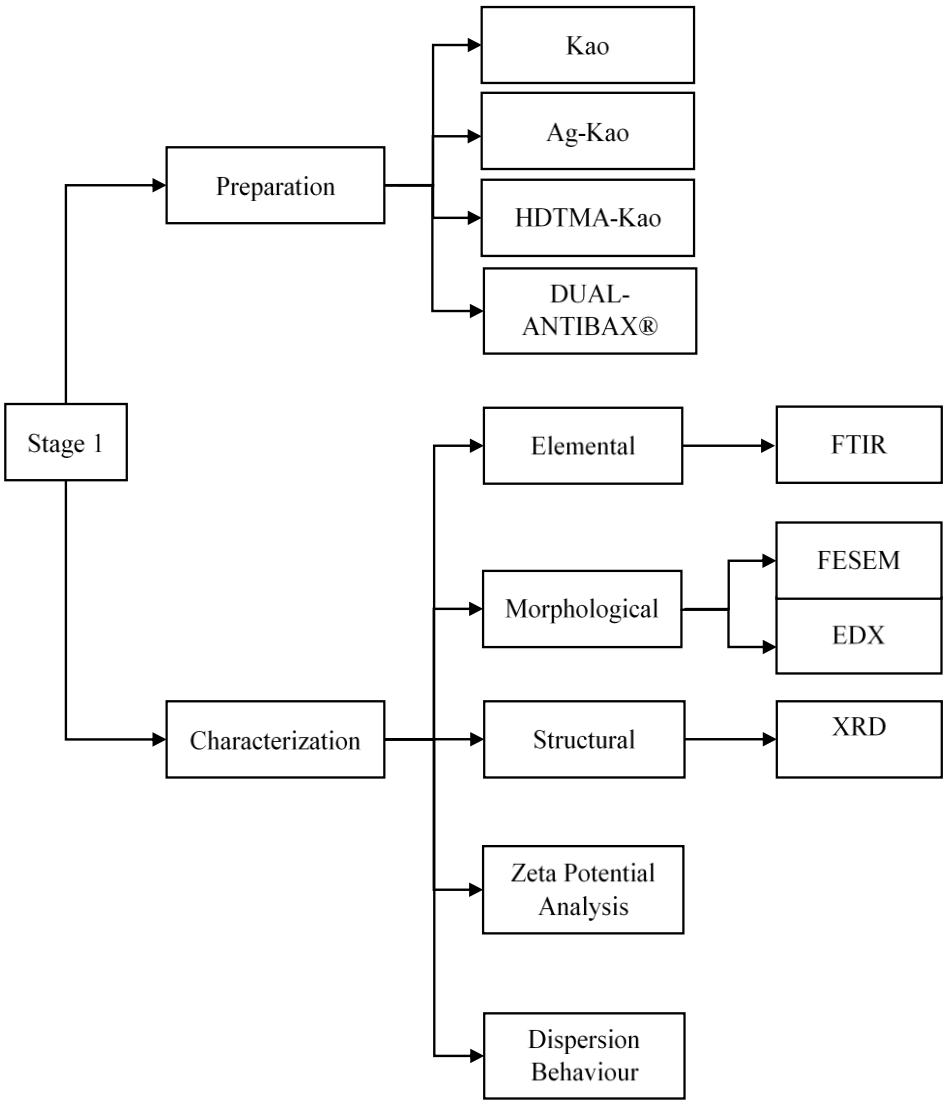

Figure 1.1: Flow diagram of the research methodology design for stage 1

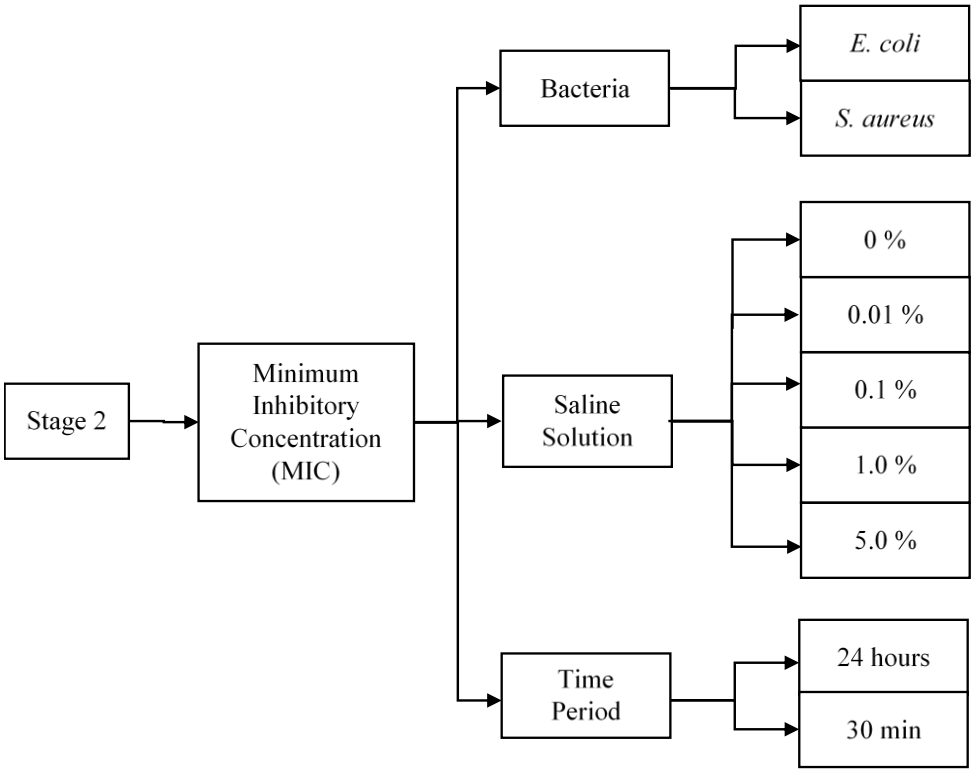

Figure 1.2: Flow diagram of the research methodology design for stage 2

1.5 Research Significance

Currently antibiotic resistance microbes have been a global threat due to their capability of causing infection. In future, this could lead to new form of diseases and complications. Therefore, new and efficient antibacterial agents are required to replace the antibiotic in order to inhibit the growth and kill the pathogens before it becomes severe. DUAL-ANTIBAX® is one of the alternative remedy that will be able to combat bacterial infections and consequently kill the bacteria.

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