SYNTHESIS AND CHARACTERIZATION OF CETYLTRIMETHYL AMMONIUM BROMIDE AND SILVER SUPPORTED NaY ZEOLITES FOR ANTIBACTERIAL APPLICATION

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This thesis is dedicated to my beloved mother, Hjh. Kalsom binti Abdul Majid, sisters and brothers.

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ABSTRACT

Microbial resistance to antibiotics and antibacterial agents and limitation of the effectiveness of the current antibacterial agent such as high cytotoxicity and short lifetime necessitates the development of advance and efficient support of the antibacterial agents. This study is about versatile application of NaY zeolite as a support for antibacterial agents cetyltrimethyl ammonium bromide (CTAB) and silver ions (Ag^+). The NaY zeolite modified with various concentrations of CTAB and Ag^+ were characterized for their structural, morphology and elemental analysis. The antibacterial activity of commercialized NaY (NaY-C) and synthesized NaY (NaY-S) zeolites modified with CTAB; and regenerated NaY (NaY-R), NaY-C and NaY-S modified with Ag^+ were investigated. The NaY-S (327.23 ± 17.70 nm) with high crystalline (crystallinity 64-88%) and high purity was synthesized with and without using pre-treated rice husk ash (RHA) by seeding and ageing techniques in hydrothermal condition. The NaY-C (700 nm) and NaY-S were modified with CTAB with varying coverage based on 50-500% of the external cation exchange capacity (ECEC) of the zeolite producing CTAB-NaY and with varying concentrations of Ag^+ (100, 600 and 900 mg/L) producing Ag-NaY. Regenerated AgY (AgY-R) zeolite was prepared by decomposition of CTAB-NaY-C (550°C, 5 hours), pre-treated with Na^+ and ion exchange with Ag^+ (100, 600 and 900 mg/L). The characterization results showed that the structure of the zeolites was preserved after calcination and modification with CTAB or Ag^+. The antibacterial activity of the modified NaY zeolites was performed against Gram-negative (*Escherichia coli* ATCC 11229 and *Pseudomonas aeruginosa* ATCC 15442) and Gram-positive (*Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212) bacteria based on disk diffusion technique (DDT) and minimum inhibitory concentration (MIC) technique in saline solution (0.9 wt.%) and distilled water. Results showed that the amount of CTAB or Ag^+ loadings affected the antibacterial activity of the samples as Gram-positive bacteria are more susceptible to CTAB-NaY, whereas Gram-negative bacteria are more susceptible to Ag-NaY. The antibacterial activities of Ag-NaY were proportional to the amount of Ag^+ loadings, whereas the size of NaY zeolites did not influence the antibacterial activity of the samples. AgY-C-900 (NaY-C zeolite with 900 mg/L initial concentration $AgNO_3$) showed optimal antibacterial activity compared to other NaY zeolites samples. The CTAB-NaY-C was regenerated to original NaY-R and reused as the support for Ag^+ with its structure remained and exhibited good antibacterial activity. Due to the good performance of the antibacterial activities of CTAB-NaY and Ag-NaY, therefore, NaY zeolite could be used as the good support of the antibacterial agents of CTAB and Ag.

ABSTRAK

Rintangan bakteria terhadap pelbagai jenis antibiotik dan agen antibakteria dan juga keberkesanan agen antibakteria yang terbatas seperti ketoksikan terhadap sel yang tinggi dan jangka hayat yang pendek telah meningkatkan keperluan penghasilan bahan penyokong untuk agen antibakteria baharu yang lebih berkesan. Kajian ini adalah mengenai penggunaan zeolit NaY yang berfungsi sebagai bahan pembawa kepada agen antibakteria setiltrimetil amonium bromida (CTAB) dan ion argentum (Ag^+). Zeolit NaY yang telah diubahsuai dengan pelbagai kepekatan CTAB dan Ag^+ telah dicirikan berdasarkan struktur, morfologi dan analisis elemen. Kajian serta perbandingan telah dijalankan ke atas aktiviti antibakteria zeolit NaY komersial (NaY-C) dan zeolit NaY yang disintesis (NaY-S) setelah diubahsuai dengan CTAB; dan zeolit NaY yang diguna semula (NaY-R), NaY-C dan NaY-S yang diubahsuai dengan Ag^+. Zeolit NaY-S dengan saiz partikel 327.23 ± 17.70 nm, kristaliniti 64-88% dan ketulenan tinggi telah disintesis dengan menggunakan abu sekam padi yang telah diproses dan juga yang belum diproses melalui teknik pembenihan dan pengeraman di dalam larutan hidroterma. Zeolit NaY-C (700 nm) dan NaY-S telah diubahsuai dengan CTAB dengan liputan yang berbeza iaitu antara 50 hingga 500% daripada kadar tukaran luar kation (ECEC) pada zeolit menghasilkan CTAB-NaY dan dengan pelbagai kepekatan Ag^+ (100, 600 dan 900 mg/L) menghasilkan Ag-NaY. Zeolit yang diguna semula dan diubahsuai dengan Ag^+ (AgY-R) dihasilkan melalui proses pengkalsinan CTAB-NaY-C pada suhu 550°C selama 5 jam, rawatan di dalam larutan Na^+ dan pertukaran ion dengan Ag^+ (100, 600 dan 900 mg/L). Struktur bahan, morfologi dan analisis elemen zeolit tersebut yang telah diubahsuai menunjukkan struktur zeolit tersebut tidak berubah selepas pengkalsinan dan diubahsuai dengan CTAB atau Ag^+. Aktiviti antibakteria zeolit yang telah diubahsuai telah dikaji terhadap bakteria Gram-negatif (*Escherichia coli* ATCC 11229 dan *Pseudomonas aeruginosa* ATCC 15442) dan Gram-positif (*Staphylococcus aureus* ATCC 6538 dan *Enterococcus faecalis* ATCC 29212) menggunakan teknik cakera penyebaran (DDT) dan penentuan kepekatan perencatan minimum (MIC) di dalam larutan garam dan air suling. Hasil kajian mendapati jumlah CTAB atau Ag^+ pada zeolit NaY menentukan aktiviti antibakteria sampel-sampel zeolit di mana Gram-positif bakteria lebih mudah dipengaruhi oleh CTAB-NaY, sementara Gram-negatif bakteria lebih mudah dipengaruhi oleh Ag-NaY. Aktiviti antibakteria Ag-NaY adalah berkadar langsung dengan jumlah Ag^+ pada sampel zeolit, di mana saiz zeolit NaY tidak mempengaruhi aktiviti antibakteria sampel-sampel zeolit. AgY-C-900 (NaY-C diubahsuai dengan $AgNO_3$ yang mempunyai kepekatan awal 900 mg/L) mempunyai kadar aktiviti antibakteria yang tertinggi berbanding sampel-sampel NaY zeolit yang lain. CTAB-NaY-C diuraikan menjadi NaY-R dan diguna semula sebagai agen pembawa Ag^+ dengan struktur zeolit tidak berubah dan menunjukkan aktiviti antibakteria yang baik. Oleh kerana CTAB-NaY dan Ag-NaY menunjukkan aktiviti antibakteria yang baik, NaY zeolit boleh digunakan sebagai bahan penyokong kepada agen antibakteria CTAB dan Ag.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

Å	-	Angstrom
°C	-	Degree Celsius
cm	-	Centimeter
cm^{-1}	-	Reciprocal centimeter (wavenumber)
g	-	Gram
h	-	Hour
kV	-	Kilo volt
L	-	Liter
M	-	Molar
m	-	Meter
mA	-	MiliAmpere
mg	-	Miligram
m^2/g	-	Meter square per gram
mg/L	-	Miligram per liter
min	-	Minute
mL	-	Mililiter
µL	-	Microliter
mm	-	Milimeter
mV	-	Milivolt
nm	-	Nanometer
rpm	-	Rotation per minute
v	-	Volume
v/v	-	Volume per volume
λ	-	Lambda (wavelength)
θ	-	Theta
°	-	Degree
%	-	Percent

<	-	Less than
±	-	Plus minus sign (confident interval)

LIST OF ABBREVIATIONS

BET	-	Brunauer, Emmett & Teller
BKC	-	Benzalkonium chloride
CEC	-	Cation exchange capacity
CFU	-	Colony forming unit
CMC	-	Critical Micelle Concentration
CPB	-	Cetylpyridinium bromide
CPY	-	Cetylpyridinium
CTAB	-	Cetyltrimethyl Ammonium Bromide
DDAB	-	Dioctyldecyldimethyl ammonium bromide
DDT	-	Disk diffusion test
DF	-	Dilution factor
DNA	-	Deoxyribonucleic Acid
DW	-	Distilled Water
ECEC	-	External Cation Exchange Capacity
EDX	-	Energy dispersive x-ray
FAU	-	Faujasite framework
FDA	-	Food and Drug Association
FESEM	-	Field emission scanning electron microscopy
FTIR	-	Fourier transform infrared
GRAS	-	Generally Regarded as Safe
HDPB	-	Hexadecylpyridinium bromide
HDTMA/HTAB	-	Hexadecyltrimethyl ammonium
ICDD	-	International Centre for Diffraction Data
ICP-OES	-	Inductively coupled plasma-optical emission spectrometry

IR	-	Infrared
IUPAC	-	International Union of Pure and Applied Chemistry
IZA	-	International Zeolite Association
LB	-	Luria-Bertani
LOI	-	Loss on Ignition
MHA	-	Mueller Hinton agar
MIC	-	Minimum inhibition concentration
MRSA	-	Methicillin resistant *Staphylococcus aureus*
NA	-	Nutrient agar
NIST	-	National Institute of Standards and Technology
OD	-	Optical density
OSDA	-	Organic structure directing agent
OTAB	-	Octyltrimethyl ammonium bromide
PDF	-	Powder Diffraction File
PFC	-	Plug Flow Combustor
ppm	-	Part per million
PTAB	-	Phenyltrimethyl ammonium bromide
PTFE	-	Polytetrafluoroethane
QAC	-	Quaternary Ammonium Compounds
QC	-	Quality Control
RHA	-	Rice husk ash
ROS	-	Reactive oxygen species
SBU	-	Secondary building unit
SD	-	Standard deviation
SDBS	-	Dodecylbenzene sodium sulfonate
SMZ	-	Surfactant Modified Zeolite
TEM	-	Transmission electron microscopy
TMAOH	-	Tetramethylammonium hydroxide
TMCS	-	Trimethylsilyl chloride
UTM	-	Universiti Teknologi Malaysia

UV-Vis	-	Ultra Violet-Visible
XRD	-	X-ray diffraction
ZP	-	Zeta potential
ZSM-5	-	Zeolite Socony Mobil No. 5

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Introduction

Nowadays, issue of antibacterial resistance to the currently used antibiotic has urged researchers to find an alternative antibacterial agent that can be used to cure the infections. New and improved ways to combat bacterial resistance must be studied and discovered to get ahead of the bacteria which is capable of evolving and acquiring resistant. Biocides are used in many areas of applications as powerful antibacterial agents which act on the bacteria in many sites. Biocides reduce the formation of antibacterial resistance because bacteria need to change their physiology at several sites in order to become resistant to the biocides. However, the use of biocides as antibacterial agents still have limitations such as the biocides (e.g. silver ions) are less stable and tend to reduce to metallic silver (Ag^0) when exposed to the heat and light which reduce its antibacterial activity. In addition, the use of high concentration biocides on the infected sites would cause cytotoxicity to the cells. Whereas, the use of low concentration biocides over a long period of time make the bacteria becoming resistance to the biocides. Thus, a support material is needed to support the antibacterial agents such as cetyltrimethyl ammonium bromide (CTAB) and silver ions to preserve their antibacterial activity in the form of ionic state and exhibit a slow release of the antibacterial agents on the infected sites.

This is where a support material, synthetic Y zeolite could play a role. The inorganic material is better than the organic material. The zeolite material has negative charges on its surface and also in its framework, which is obtained from aluminium in the zeolite backbone. This condition makes the zeolite available for the attachment of the positively charged ion/compound such as sodium ions to stabilize the negatively charged zeolite. In this study, CTAB and silver ions were loaded onto the NaY zeolite through ion exchange with sodium ions which present in the zeolite framework. For the large size of CTAB head, they only attached on the zeolite surfaces through ion exchange of CTAB molecules with sodium ions situated on the zeolite surface. Meanwhile, due to the small size, silver ions able to be ion exchanged with sodium ions on the zeolite surface and also in the zeolite framework. Surfactant modified zeolite and Ag-modified zeolite exhibited a good antibacterial property. The synthetic zeolite is better than the natural zeolite as it is pure and its structure can be engineered during the synthesis. However, synthetic zeolite has been synthesized using expensive chemical reagents which made the synthetic zeolite costly and eventually, limiting its application as a support material for the antibacterial agents in household and hygienic products.

In this study, the synthetic zeolite (NaY zeolite) was synthesized using rice husk ash (RHA) as the silica source and then, surfactant modified zeolite (CTAB-modified NaY zeolite) can be regenerated to the original NaY zeolite and again reused as a support material of silver ions (regenerated AgY zeolite). The study proves that NaY zeolite is a good support material of the antibacterial agents of CTAB and silver ions. This interdisciplinary research requires in-depth knowledge or understanding in inorganic chemistry in order to produce inorganic materials, physical chemistry to understand the physical characteristics of materials and their applications, and also biological knowledge to understand the role and effect of compounds as antibacterial agents. In this way, early prevention of bacterial infection can be done. New antibacterial agents will continuously emerge as long as infections and disease exist within the communities where people are always looking for better curing approach. Therefore, this study shows an effort that can be done to improve the quality of human health and life with the aim of developing a better antibacterial agent that employs an improved mechanism in its antibacterial action.

1.2 Problem Statements

The development of new types of antibacterial agents has been important nowadays since the emergence of bacteria resistant to antibiotics (Tortora *et al*., 2007). This issue is of great concern to the medical microbiologist or microbiologist as reported in numerous review papers (Cantas *et al*., 2013; Crouch *et al*., 2015; Schwarz *et al*., 2017; Leangapichart *et al*., 2017; Zaman *et al*., 2017). A major global health concern, for example, antimicrobial resistance (AMR) has caused death from the current estimate about 700,000 lives per year and will increase to ten million lives annually by 2050 which cost US$100 trillion (Brogan and Mossialos, 2016; O'Neill, 2016). Different bacterial species that have been once susceptible to several different classes of antibiotics have now acquired an array of unique resistance mechanisms. Currently, bacterial resistance to the available antibacterial agents becomes the major obstacle in the treatment of infectious diseases. Antimicrobial resistance becomes the global health problem which increases the morbidity, mortality, and causes serious economic, social, and political implication. As a result, this problem has caused treatment failures of the healthcare institutions (Tenover, 2006). The cost of treating the disease also increases as several therapeutic agents need to be applied to the infected sites, and the infected people should stay longer in the hospital. Antibacterial resistance is a natural biological phenomenon as a response from microbes such as bacteria, parasites, fungi and viruses to the antimicrobial agents (Sharma *et al*., 2005). Therefore, alternative strategies of the conventional antibiotic therapy are greatly desired (Zeng *et al*., 2008). One strategy to avoid this is by using alternative therapeutic agents from biocides such as a Quaternary ammonium compound, (CTAB) and silver ions supported onto NaY zeolite.

Antibiotic is an organic substance which is naturally (penicillin) and synthetically produced (sulfonamide) that attacks the bacteria at the specific site of the bacteria, and used in low concentration. An antibiotic is basically used inside the body of the host (Tortora *et al*., 2007). As the pathogenic bacteria become resistant to the current antibiotic, other alternative therapeutic agents must be sought. Biocides as antibacterial agent have a broad spectrum capability which attack several sites of the bacteria, thus difficult to cause bacterial resistance to biocides (Tortora *et al*., 2007). Although bacterial resistance to biocides occur, such probability is low as the bacteria need to generate several processes to cope with biocides. The current uses of biocides with the inclusion of ethanol, silver nitrate and surfactant in the form of solution (elutable) have a few drawbacks. For example, ethanol easily evaporates at room temperature, cause skin irritation and inflammable (Tilton and Kauffman, 2004). Silver nitrate solution less stable, easily oxidized when exposed to light (Carolina *et al*., 2014) and heat (Kittler *et al*., 2010), and react with chloride ions in solution forming silver chloride, which reduced the antibacterial properties of the agent (Swathy *et al*., 2014). High concentration of the agents would cause cytotoxicity. Basic requirements of novel biocidal materials are (1) facile synthesis, (2) long term stability, (3) water insolubility, (4) non-toxicity, and (5) broad spectrum biocidal over a short contact time (Kenawy *et al*., 2007). In order to produce a good antibacterial material, the antibacterial agents must release enough antibacterial agents in certain time. Thus, support materials are required to support the antibacterial agents to make them stable (not affected by the environment), and slow release of the agent in a long period of time.

The organic materials that are used as supports are cellulose, agarose and sepharose. However, these materials have drawbacks; for instance, they are easily affected by temperature and pressure, as well as expensive compared to the zeolite (Sakaguichi *et al*., 2005). In addition, the organic materials easily deteriorate with time, thus not able to support the antibacterial agents for long periods. The inorganic antibacterial materials are better than the organic antibacterial materials with the properties such as thermal resistance, safe for users, high chemical stability, long lasting action period and others (Dolic *et al*., 2015). Other inorganic materials used as support materials are natural zeolites and clays. However, both of them naturally exist in the environment, and contain impurities of other minerals (Sherman, 1999) and various metals (Breck, 1974). These impurities make the materials inhomogeneous, and thus lower the efficiency of the materials as support material. The metals which present in these materials would cause cytotoxicity causing difficulty to predict the result of the antibacterial materials. Natural zeolites (Payra and Dutta, 2004) and clays (Breck, 1974) have a lower surface area and ion exchange capacity (CEC). Clay materials have a two-dimensional structure which causes it to easily expand when the water is adsorbed (Breck, 1974), and thus would clog the pore when applied to water filter (Herrera *et al*., 2000). In contrary, zeolite is a three-dimensional structure, thus rigid and stable (Breck, 1974), suitable to be a good support material for the antibacterial agents. However, as natural zeolites and clays have the adsorbent properties and abundantly available on earth, they can be used for the environment purposes such as in wastewater treatment due to its high ion exchange capacities, cost effectiveness and environmental compatibility.

Synthetic zeolite was chosen as the support material of the antibacterial agents in this study because the synthetic zeolite is pure, and able to predict the reaction of the material concisely. Moreover, the structure, purity, chemical composition and porosity can be engineered during synthesis (Breck, 1974). Synthetic zeolite has high purity and uniformity, molecular size pores, regular crystal structures, large internal pore volumes, high cation exchange capacity (CEC) and sorptive capacity, high surface area, negative surface charge, low or null toxicity, chemical inertness and diverse framework chemical compositions (Sherman, 1999; Yusof and Malek, 2009). Low Si/Al ratio zeolite possesses high adsorbent capacity for polar molecules and provides more exchange sites. The NaY zeolite (in powder form), a low Si/Al ratio zeolite which is less than 14, has large cation exchange capacity, large pore volume and high crystalline suitable to be used as the support material of the antibacterial agents of CTAB and silver (Hagiwara *et al*., 1990). The drawback of synthetic zeolite is that they were synthesized using expensive standard chemical reagents (Matti and Surchi, 2014), therefore the production cost of the materials is high which limits the use of the materials for household and hygienic products. In this study, an agricultural waste (e.g. rice husk ash) was used as the silica source to synthesize small crystallite size of NaY zeolite (Rahman *et al*., 2009). Besides, the CTAB-modified NaY-C zeolite (surfactant modified zeolite) was regenerated to original NaY (regenerated NaY) by thermal treatment (calcination) and reused as the support of silver ions (regenerated AgY).

Zeolite is an inorganic crystalline material with three-dimensional framework structure consists of aluminosilicates as its backbone, comprising cations and water molecules in its framework (Breck, 1974). The cations are from a group I or group II in the periodic table (Na^+, K^+, Mg^{2+}, Ca^{2+}) located in the zeolite framework to stabilize the negative charges of exchangeable sites (Breck, 1974). The cations are mobile and can be exchanged with other cations present in the solution while the intra-crystalline zeolitic water can be removed reversibly (Breck, 1974). For instance, cationic surfactant QAC such as CTAB molecules having positively charge surfactant head and silver ions (Ag^+) could be adsorbed onto zeolite through ion exchange with the cations which are present on zeolite surface and in the zeolite frameworks. In terms of the attachment of surfactant molecules adsorbed on zeolite, the surfactant molecules are too large to enter the small pore of zeolite and they are attached on zeolite surfaces. Specifically, cationic surfactant CTAB molecules having a head diameter of 0.694 nm (Rozic *et al*., 2009) are unable to penetrate the average pore diameter of NaY zeolite (0.74 nm) (Nezamzadeh-Ejhieh and Badri, 2011b). As a result, they would occupy the exchangeable active sites at the exterior of NaY zeolite framework. On the other hand, the small size of cationic silver ions would be adsorbed on the zeolite surface as well as inside its framework (Fonseca and Neves, 2013). The conventional antibacterial agents can be improved by immobilizing the biocides in the supports and then release a low concentration of biocides in the long term (He *et al*., 2006; O'Neill *et al*., 2006; Bedi *et al*., 2012). The flexibility of zeolites (e.g. NaY zeolite) as the adsorbent materials could reduce the cost of the preparation. NaY zeolite possesses a cubicle structure (Breck, 1974), which is less harmful to the body. Furthermore, zeolite has been approved by the Food and Drug Association (FDA) as Generally Regarded as Safe (GRAS). Therefore, it is possible to use CTAB- (McDonnell and Russell, 1999) or Ag-modified NaY zeolites (Klasen, 2000) as an antiseptic agent for skin and for the treatment of wound infections.

The incorporation of silver ions in the suitable support material could solve the problem as silver ions would slowly release into the solution containing bacteria (Matsumura *et al*., 2003). According to Ferreira *et al*. (2015), silver ions are preserved in its ionic state in AgY zeolite. Moreover, Lalueza *et al*. (2010) claimed that silver ions in Ag-zeolite would release into the solution if only the Ag^+ sites are exchanged with other cationic ions in the solution that would take Ag^+ place and pump out Ag^+. In addition, Matsumura *et al*. (2003) revealed that in water condition, Ag^+ may be released from zeolite when bacterial cells are present. Thus, the incorporation of Ag^+ in zeolite would preserve the silver remained in ionic form (Ag^+) (Ferreira *et al*., 2015) and slow down the release of Ag^+ progressively into the medium containing bacteria (Matsumura *et al*., 2003). On the other hand, the incorporation of Ag^+ into zeolite could solve the problem occurred with $AgNO_3$ which is inconvenient for handling as well as can be used for limited purposes (Hagiwara *et al*., 1990). Compared to other transition metals (Zn, Cu), silver ions display higher antibacterial activity (Malachova *et al*., 2011) and zeolites possess better selectivity for Ag than for Zn and Cu (Top and Ulku, 2004). Furthermore, Ag-zeolite displays similar antibacterial activity to $AgNO_3$ (Matsumura *et al*., 2003), and it is expected that the incorporation of Ag^+ in zeolite has not reduced the antibacterial activity of Ag^+.

Silver ions have a broad spectrum antimicrobial properties, depicting high thermal stability and low volatility, displaying cytotoxicity to animal cells (dependent of the silver concentration), relatively inert and safe (Ferreira *et al*., 2015). Despite precious properties of silver as the antimicrobial agents, Ag-based products generally have two main drawbacks: (1) bacterial resistance to silver (Silver *et al*., 2006); and (2) formation of insoluble precipitates (e.g. AgCl or Ag_2S) which occurs due to the reaction of Ag^+ from Ag-zeolite with electrolytes (e.g. chloride and sulfur anions) in bacterial solution (Cowan *et al*., 2003). This will reduce the antibacterial activity of Ag-zeolite (De la rosa-gomez *et al*., 2008). Bacterial resistance to silver can occur when the bacterium is rendered in a sublethal concentration of silver for long periods of time (Chopra, 2007). This problem can be solved by increasing the concentration of silver in solution for an instant antibacterial activity (Chopra, 2007). As in this study, Ag-modified NaY zeolite was prepared using different particle size and surface area and thus it is expected that Ag-modified NaY with smaller particle size and larger surface area would release more silver ions compared to Ag-modified NaY with larger particle size and lower surface area, and exhibit an instant antibacterial activity towards the tested bacteria.

Only Ag^+ has the antibacterial property (Inoue and Kanzaki, 1997). Exposure to high concentration of silver would cause argyria, a skin condition where the color turns grey caused by accumulation of silver (Baker *et al*., 2011). By loading Ag^+ onto the zeolite, the Ag state can be preserved as Ag^+ in the zeolite (Ferreira *et al*., 2012), and only released into the solution when other cationic ion has exchanged with Ag^+ in zeolite (Lalueza *et al*., 2010), and only when the bacteria are present (Matsumura *et al*., 2003). Thus, the antibacterial activity of Ag-modified zeolites can last longer and could reduce the cytotoxicity effect of silver. Although Ag is an expensive metal, but due to the oligodynamic effect of the metal, only a small amount of metal is needed in order to exhibit high antibacterial property (Shrestha *et al*., 2009). Low concentration of Ag^+ can be preserved in the zeolite framework in the form of Ag^+ (Ferreira *et al*., 2012). High concentration of Ag^+ loaded onto zeolite could cause the loosely bound Ag^+ on the zeolite surface reduced to Ag^0 upon light or heat exposure (Saint-Cricq *et al*., 2012).

Quaternary ammonium compounds (QACs) cationic surfactant having number of C with C8 to C18 in the hydrocarbon tail have the antibacterial properties. For instance, CTAB with C16 of hydrocarbon tail has a broad spectrum of antibacterial properties effective against Gram-negative and Gram-positive bacteria (Dizman *et al*., 2007). Additionally, Ag has broad spectrum antimicrobial properties which are effective against Gram-negative and Gram-positive bacteria (Mintova *et al*., 2015). Both QACs and cationic metal Ag^+ have a broad spectrum activity. They attack the pathogenic bacteria on several sites, and subsequently decrease the probability of bacterial resistance. Surfactant can be used as skin antiseptics and disinfectants (Dizman *et al*., 2007), while Ag is used as an antibacterial agent for burns (Nherera *et al*., 2017). They are applied to the infected sites or on surfaces in the form of solution (elutable biocide). Elutable biocides have some disadvantages such as short time effectiveness; thus, need to be applied frequently on the infected sites and possibly to cause toxicity to the surrounding tissues (Fonseca and Neves, 2013).

In order to produce effective Ag-modified zeolite antibacterial material, only a low concentration of Ag is needed. Also, the attachment of surfactant in the forms of partial monolayer and monolayer coverage on the zeolite through ion exchange reaction with the Na^+ on the zeolite surface (Vidal *et al*., 2012) is via electrostatic attraction. This is a strong bonding and almost irreversible (Ozdemir *et al*., 2013). Due to that, surfactant modified zeolite (partial monolayer and monolayer coverage) can become a long lasting antibacterial agent (He *et al*., 2006).

The NaY zeolites were synthesized using RHA as the silica source. RHA contains more than 90% silica (Yusof *et al*., 2010) and the only agricultural waste that has high silica content in dry form (Jain *et al*., 1994). This rice husk material is decomposed by burning it on the field (Yalcin and Serinc, 2001) and by rotting (Rahman *et al*., 2009) which could affect the environment and health. Thus, by using RHA as the silica source in zeolite synthesis, beneficial products (e.g. zeolites) can be produced from the agricultural waste and also can solve the problems which occur due to the deposited of rice husk on the field (Rahman *et al*., 2009).

1.3 Objectives of the Research

Three main objectives of the research are listed below:

1. To synthesize and characterize highly pure small crystallite size of NaY zeolites using rice husk ash with variation of pre-treatments.
2. To prepare and characterize CTAB-modified NaY, regenerated AgY and Ag-modified NaY zeolites.
3. To study the antibacterial activity of CTAB-modified NaY, regenerated AgY and Ag-modified NaY zeolites against Gram-negative and Gram-positive bacteria.

1.4 Scope of the Research

This research can be divided into four scopes. The first scope of the work encompasses the synthesis of small crystallite size of NaY zeolites with similar method of synthesis of microsized NaY zeolite (Yusof *et al*., 2010) with modification in order to reduce particle size of zeolite and using different pre-treatments method for rice husk ash as the silica source. The synthesis process will be carried out in a hydrothermal condition with 5% seed gel, static aging at room temperature (25°C, 24 hours), low crystallization temperature (90°C, 22 hours) using an organic template-free technique.

The second scope of the work encompasses the preparation of the CTAB-modified NaY zeolites using different surfactant coverage (e.g. 0.5-5.0) of the External Cation Exchange Capacity (ECEC) of each NaY zeolite (NaY-C: 0.53 meq/g (Yusof and Malek, 2009), NaY-S: 1.15 meq/g), followed by characterization analysis (e.g. structural, morphological, elemental analysis and several physicochemical properties) and antibacterial testing (e.g. disk diffusion (Kirby-Bauer); and Minimum Inhibitory Concentration (MIC) in a saline solution and distilled water of the samples against Gram-negative (*Escherichia coli* ATCC 11229 and *Pseudomonas aeruginosa* ATCC 15442) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212). The structural stability of CTAB-modified NaY zeolites and the presence of surfactant molecules on the NaY zeolites also will be investigated.

The third scope of the work is the investigation of the flexibility of NaY-C zeolite as the antibacterial materials as its structural stability will be evaluated by modification (e.g. CTAB), thermal treatment (e.g., calcination 550°C, 5 hours) and modification with Ag^+ ([$AgNO_3$]: 100, 600 and 900 mg/L). Then, the regenerated AgY zeolite samples obtained will be tested for their antibacterial activity against Gram-negative and Gram-positive bacteria as mentioned previously. This study will be carried out to investigate the effect of the treatments (e.g., modifications and thermal treatments) on the structural and antibacterial activity of regenerated AgY zeolite samples.

Finally, the fourth scope of the work encompasses preparation of Ag-modified NaY zeolites using different particle size and Si/Al ratio of NaY zeolites (NaY-C: 700 nm (Ferreira *et al*., 2015) (Si/Al ratio: 4.37), NaY-S: 327.23 ± 17.70 nm (Si/Al ratio: 2.03)) loaded with similar initial concentrations of silver ([$AgNO_3$]: 100, 600 and 900 mg/L), followed by characterizations and antibacterial testing using methods and bacteria as mentioned previously. Due to the lower initial concentrations of silver used, it is postulated that almost all silver ions in the solutions would occupy the negative exchange sites of both NaY zeolites. This study has been carried out to investigate the effect of the zeolite particle size and surface area on the antibacterial activity of Ag-modified NaY zeolites. The mechanism of the antibacterial activities of CTAB- and Ag-modified NaY zeolites will be evaluated by morphological study (e.g., FESEM and Gram stain), viability study (post-MIC) as well as the release of CTAB and Ag in the saline solution and distilled water.

1.5 Significance of Research

To the best of our knowledge, there are four researches have been done on the antibacterial properties of silver nanozeolites (Tosheva *et al*., 2012; Dong *et al*., 2014; Wu *et al*., 2015; Wu *et al*., 2017). One of the researches studied the biomedical properties of silver nanozeolite (Kaur *et al*., 2015), while other research studied the electrocatalyst properties of Ag-loaded ZSM-5 nanozeolites which was synthesized from bagasse (Rostami *et al*., 2017). In a recent study based on the application of Ag^+/Ag^0 loaded onto the nanozeolite, it was used as a coating material on a membrane surface as the Ag-nanozeolite was grafted on a membrane with polyvinyl alcohol (PVA) and polydopamine (PDA) (Wu *et al*., 2017). Another research was carried out by Wu *et al*. (2015) based on the incorporation of Ag-nanozeolites onto the commercial polyamide nanofiltration membrane to prevent biofouling in long term membrane applications. Tosheva and her co-workers (Tosheva *et al*., 2012) studied the antibacterial properties of Ag and Cu loaded nano- and micro-sized FAU-type zeolite, but the nanozeolite used in their study was synthesized using different methods ((1) preparation of highly reactive gels at room temperature (Valtchev and Bozhilov, 2004); (2) using a three-stage temperature control synthesis procedure (Huang *et al*., 2010); and (3) using 2^3 factorial methods for optimization of the experimental conditions (Kim *et al*., 2008)), and they used different silica source which was fly ash in the preparation of the materials. Thus, it is expected that the zeolite properties would be varied from the NaY zeolite obtained from rice husk ash. Besides, they were using different concentrations of silver ions and different antibacterial testing from this study. Meanwhile, Dong *et al*. (2014) studied the antibacterial properties of EMT-type nanozeolite comparing the antibacterial properties of two types of silver Ag^+-EMT and Ag^0-EMT against *E. coli* ATCC 8739. In addition, a study reported by Kaur *et al*. (2015) on biomineralization of hydroxyapatite in silver ion-exchanged nanocrystalline ZSM-5 zeolite using simulated body fluid found that the materials were considerable potential for biomedical applications such as for bone implant. Rostami *et al*. (2017), on the other hand, studied the application of Ag-loaded ZSM-5 nanozeolites synthesized from the bagasse as electrocatalyst in electrode, as the Ag-loaded zeolite was added onto the carbon paste electrode (Ag/ZSM-5/CPE) used for electrooxidation of oxalic acid.

Surfactant modified nanozeolite mainly clinoptilolite nano-particles were used as the active component of Cr(VI) selective electrode (Nezamzadeh-Ejhieh and Shahanshahi, 2013), adsorbent materials of Pb(II) from aqueous solution (Anari-Anaraki and Nezamzadeh-Ejhieh, 2015) and carrier of cephalexin drug delivery (Nezamzadeh-Ejhieh and Tavakoli-Ghinani, 2014). None of the research used surfactant modified small crystallite size of zeolite especially CTAB-modified small crystallite size of NaY zeolite as antibacterial materials. In addition, the study of the antibacterial activity of regenerated AgY zeolite obtained from regeneration of CTAB-modified NaY zeolite (surfactant modified zeolite) is readily new.

Immobilizing the antibacterial agents (CTAB, Ag) onto a support material could improve the properties of the antibacterial agents (e.g. prevent cytotoxicity and prolonged the antibacterial activity of the agents). In this study, different particle size of NaY zeolites (commercial and synthesized NaY) was used as the support material for organic and inorganic antibacterial agents (CTAB-modified NaY and Ag-modified NaY). Also, the CTAB-modified NaY (surfactant modified zeolite) was regenerated to original NaY (regenerated NaY) by thermal treatment, and followed by modification with silver ions forming regenerated AgY zeolite. Therefore, this research is significantly important in developing new and improved antibacterial agents in order to cope with antibiotic resistance problems and ineffective antibacterial agents.

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