EFFECT OF SILVER-BASED PRODUCTS ON SIMULATED BODY FLUIDS

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I would like to dedicate this thesis to special people in my life:

I am ever grateful to my beloved husband, Mehdi, for his encouragement, patience and affection.

My genuine appreciation goes to my beloved parents, Ahmad and Parivash who always believe in their daughter and never let me down. Without their f supports even a word of this thesis has not been written

To my brother Mohamad and my sister Azita for their inspiration and love.

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ABSTRACT

Zeolites A, Y, clinoptilolite, bentonite and kaolinite were applied as a carrier material to attach antibacterial agents of silver ion (Ag^+) (Silver based product (Ag-BP)). They were analyzed by EDX and FTIR spectroscopy and showed that the Ag^+ attached on the zeolites and clays while the main structure of them did not change significantly. The effects of Ag-BP on simulated body fluid (SBF) and simulated gastric fluid (SGF) were studied in different concentrations. The chemical framework variations of Ag-BP were analyzed by FTIR spectroscopy after contact with SBF and SGF. In FTIR, the most important structural peaks were same as those parents represent Si-O-Si and Si-O-Al bands. The antibacterial activity of Ag-BP was studied by Kirby-Bauer method against *Escherichia coli* and the inhibitory zones were found in this assay showing that they have antibacterial activity. Ag-BP had more structural variation after contacted in SGF (pH: 1.2) than SBF (pH: 7.4). With increasing concentration of adding Ag-BP to SGF and SBF, more changes were occurred on their structures. Strong acidic condition in SGF created an acidic attack to Si-O-Si and Si-O-Al bands in main frameworks of SBP. Due to this attack, some bonds such as Si-O-Si and Si-O-Al in Ag-BP were changed and caused to release their chemical agents in SGF. Interactions between SBF and Ag-BP were insignificant because the pH of SBF was not changed after addition of SBPs in SBF. In conclusion, certain structural changes were observed in the framework of Ag-BP and SGF when adding together while these changes were not significant for Ag-BP in SBF.

ABSTRAK

Zeolit A, Y, klinoptilolit, bentonit dan kaolinit digunakan sebagai bahan pembawa untuk memegang ejen antibakteria ion perak (Ag^+) (Produk berasaskan perak (SBPs)). Sampel-sampel telah dicirikan menggunakan penganalisis EDX and spektroskopi FTIR dan ia menunjukkan bahawa Ag dipegang oleh zeolit dan tanah liat manakala struktur utama bahan tersebut tidah berubah. Kesan SBPs pada simulasi bendalir tubuh (SBF) dan simulasi cecair gastrik (SGF) dikaji dalam kepekatan yang berbeza. Perubahan kerangka kimia SBPs dianalisis menggunakan FTIR selepas ditambah dengan SBF dan SGF. Melalui FTIR, puncak struktur yang paling penting sama dengan bahan asal mewakili Si-O-Si and Si-O-Al. Aktiviti antibakteria oleh SBPs dikaji melalui kaedah Kirby-Bauer terhadap *Escherichia coli* dan zon perencatan yang diperolehi menunjukkan yang SBPs mempunyai aktiviti antibakteria. SBPs menunjukkan perubahan struktur selepas ditambahkan kedalam SGF (pH: 1.2) dibandingkan dengan SBF (pH: 7.4). Dengan pertambahan kepekatan SBPs terhadap SGF dan SBF, banyak perubahan yang berlaku terhadap strukturnya. Keadaan keasidan yang tinggi pada SGF menyebabkan serangan keasidan kepada Si-O-Si dan Si-O-Al pada kerangka utama SBPs. Disebabkan serangan ini, sebahagian rangkaian dalam SBPs berubah dan menyebabkan pelepasan bahan kimia kedalam SGF. Interaksi antara SBF dan SBPs tidak begitu ketara kerana pH SBF tidak berubah. Sebagai kesimpulan, perubahan pada sebahagian struktur dilihat pada kerangka SBPs dan SGF apabila ianya ditambahkan bersama manakala perubahan tidak begitu ketara bagi SBP dalam SBF.

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LIST OF ABBREVIATIONS AND SYMBOLS

Ag	Silver
Al	alumina
$AgNo_3$	silver nitrate
°C	Degree Centigrade Celsius
$CaCl_2$	calcium chloride
$C_6H_{10}O_5$	3.Deoxyglucosone
Ca	Calcium
Cd	Cadmium
Cl	chloride
Cm^{-1}	Centimetre (for energy unit)
CO_2	Carbon dioxide
C-O-C	Carbon- Oxygen- Carbon
CONAB	National Food Supply Agency in brazil
Cr(VI)	Chromium six
CFU	Colony Forming Unit
Cu	Copper
E. coli	*Escherichia coli*
ECF	*extracellular fluid*
EDX	Energy Dispersive X-Ray
FTIR	Fourier Transform Infrared Spectroscopy
g	Gram
$(CH_2OH)_3CNH_2$	Hydroxymethyl-aminomethane
H	Hour
H_2O	Dihydrogen oxide(water)

H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid
HCl	Hydrogen chloride acid
HNO_3	Nitric acid
HCO_3	Carbonic Acid
IR	Infrared spectroscopy
$K_2HPO_4.3H_2O$	dipotassium phosphate anhydrate
KCl	potassium chloride
KOH	Potassium hydroxide
MIC	Minimum Inhibition Concentration
$MgCl_2.6H_2O$	chloride hexahydrate
Mg	Magnesium
ml	Milliliter
mm	Millimetre
Mmol/gr	Millimole/ gram
Mol\L	Moll/litre
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
NaOH	Sodium hydroxide
Na^+	Sodium Possitive Ion
OH group	Hydroxyl group
Pb	Lead
rpm	Rotations per minute
Si	silicon
SBF	Simulated Body Fluid
SGF	Simulated Gastric fluid
SBPs	Silver Based Products
SO_4	Sulfate
Si-O-Al	Silicon-Oxygen-Aluminium
Si-O-Si	Silicon-Oxygen-Silicon
UV	Ultra Violet

w/w	weight/weight
Wt %	Weight percent

CHAPTER 1

INTRODUCTION

1.1 Background Information

Antibiotic is a type of medications that functions as an antibacterial. The first antibiotic was penicillin discovered by Alexander Fleming in 1929. Antibiotics such as streptomycin, chloramphenicol, and tetracycline were discovered more recently. Sometimes, antibiotics may fail to prevent from the growth of bacteria. It is due to the fact that some bacteria have enough genetic information on how to survive in the presence of antibiotics or have mutation in gene that makes the treatment of bacterial infections more complicated (White and McDermott, 2001). A problematic area for medical microbiologists is resistance of some bacteria against antibiotics or antimicrobial agents. Therefore, it is essential to find a new method that could replace the antibiotics for treating bacterial infections. To solve this problem, many researchers recently have attempted to find effective antimicrobial agents free of resistance and low cost, which has led to the use of Ag-based antibacterial agents (Jun *et al.*,2007).

Silver (signified chemically as Ag) that is commonly known as a heavy metal is widely used in many products in our daily life. It is generally a solid material with various chemical and physical properties. Different unique features of silver make it proper to be applied in various industrial and medical cases. In optical and electrical industries, the silver positive ions are used. Silica core-silver shell nanoparticles (silver nanoshells) produces a silver outer layer was used in UV−vis spectroscopy and transmission electron microscopy (Jackson and Halas, 2001). Additionally, in the medical field, silver could be employed as an antimicrobial agents. They can react with the critical bacterial enzymes in order to neutralize their function, disrupt the electron transport system, and attach to their DNA (Baker *et al.*, 2003). Silver ions also can modify the construction of the cell envelope of the bacteria. Among metal ions, silver ion has the maximum antimicrobial action, and a range of silver complexes have been used as agents for treatment (Kawahara *et al.*, 2000). In the current study, silver nitrate ($AgNo_3$) has been used in the preparation of silver based products (SBPs) because of its higher solubility in water and other aqueous solutions in comparison with other silver compounds.

Silver can be harmful for human health. Prolonged exposure to that metal can cause some illnesses like argyria (reversible pigmentation of the skin) and/or argyrosis (reversible pigmentation of the eyes). The affected area becomes bluish-gray or ash gray, and it is most prominent in areas of the body exposed to sunlight (Drake and Hazelwood, 2005). Also, silver particles have been shown to have some of cytotoxicity effects in some human cells, and they possess a high affinity for the thiol groups in the liver and reduce glutathione, and consequently, it leads to depleting the amount of glutathione available for biochemical pathways. Reduced glutathione plays an important role in maintaining proper structure and function of red blood cells, as well as eliminating organic peroxides (Baldi and Nucci, 1988).

Due to particular characteristics of silver, the silver-based products (SBPs) have gained specific attention in various medical, pharmaceutical, industrial, and engineering fields. Today, many well-known SBPs are globally produced, including silver based colours, silver nanoparticles, silver sulfadiazine, silver nitrate, silver chloride, silver zeolites, and silver clays. SBPs are potentially applicable in producing the antibacterial agents. Silver-loaded zeolite X shows antimicrobial action, thus, it can be used as antibacterial agent against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Kwakye-Awuah *et al.*, 2008). The silver-based nanoclay demonstrated strong antibacterial activity against *Salmonella* spp.(Busolo *et al.*, 2010). The antimicrobial properties of silver-modified montmorillonites are also examined in growth inhibition of *Escherichia coli (E. coli)* and the results proved its positive effects (Magana *et al.*, 2008).

There are much more to be said about the practical applications of SBPs in engineering and industries. Applications such as the *in vitro* efficacy of silver sulfadiazine and chlorhexidine against bacteria usually found in burn wounds and some kinds of silver-based dressings that are commercially available can be mentioned as follow. Aquacel¯Ag , Contreet Foam which are used for wounds infection treatment (Burd *et al.*, 2007), ultrasensitive silver-based color which is applied for staining of polypeptides in polyacrylamide gels (Sammons *et al.*, 1981), using silverized activated carbon filters beside the ion-exchange resins (Gupta, 1998) for water purification units and distribution systems of hospitals and hotels to manage contagious agents (Li *et al.*, 2005), using polylactic acid (PLA) biocomposites which obtained by solvent casting, with a new silver-based antibacterial layered silicate additive which is suitable for food packaging requests and etc.

One of the supporting material for silver ion and other cations is zeolite that has a porous structure and consists of hydrated aluminosilicates. It is classified into two major categories: natural and synthetic zeolites. The former includes chabazite, mordenite, and Clinoptilolite; whereas zeolite Y, zeolite X and zeolite A are known as synthetic zeolites (Kaduk and Faber, 1995). Zeolites are one of the most popular molecular sieving with high ion-exchanged capacity (Takaishi *et al.*, 1975). Zeolites do not have antibacterial activity but it can be induced with adding the silver ions and surfactant molecules (Rivera *et al*, 2000). Zeolites that are structurely modified with silver are capable to function as an antibacterial agent. Those matrixes could be appropriately used as carriers because of their high ion exchange capacity, high surface area, negative surface charge, chemical inertness, and low toxicity. Zeolites can play a significant role as a supportive material for silver ions to create an alternative substance to overcome the antibiotic resistance problem through improving some antibacterial agents' abilities.

Another supporting material is natural clay minerals that are widely used because of their low expense and abundance. Clay materials are important as adsorbents because of their unique framework which have eminent sorption specifications and high capacity for ion exchange. Clay minerals have a layered structure of silica (Si), alumina (Al), and water. They can be divided in several groups such as montmorillonite group , kaolinite group, chlorite group, and illite group, as well as bentonite clay which is a marketable material made up of montmorillonite and smectite clay (Nayak and Singh, 2007). Clays like zeolites provide a physically stable surface for silver nanoparticles and silver ions to be a supporting material for them(Etris and Cappel, 2003). Silver ion is released from zeolites and clays in the infectious area (Matsumura *et al*, 2003), therefore, silver-modified mineral clay can be used as an antibacterial agent. Despite widespread use of silver-based products, there are very few studies on the effects of them on human organs and fluids such as body, gastric, and intestine fluids.

Since 1987, the literature has witnessed a huge and sudden increase in the application of Simulated Body Fluid (SBF) for bioactivity testing purposes. The human body fluids are divided into two parts: (a) the *extracellular fluid* (ECF) including roughly one-third of water of the human body, and (b) *intracellular fluid* with two-thirds of body water. The body fluid simulated for the experiment in the current study consists of different ions like K^+, HCO_3, Na^+, Cl^-, Mg^{+2}, Ca^{+2}, SO_4, and HPO_4 (Marques *et al.*, 2011). Moreover, the simulated body fluid could be applied in evaluating the impact of drug on the human body fluid and providing an environment the same as the human body in order to estimate mutual influences of these fluids with drugs or materials.

Some studies have reported the usefulness of the SBF in testing bioactivity. (Kokubo and Takadama, 2006). Another body examined in this study in terms of the SBPs is the simulated gastric fluid (SGF) with a pH of below 2, during fasting state. It is noticeable that its pH can be ranged from 1 up to 7. 5 after food intaking. Based on the meal contents, the fed-state gastric pH will rise to values ranged between 4 and 7 (Marques *et al.*, 2011).

Similar to SBF, the simulated gastric fluid was initially developed for estimating the effect of drug on the human gastric fluid and providing a situation similar to the real human stomach. It should be performed in order to evaluate the reciprocal impacts of SGF with the structures of the drugs or materials.

A simulated gastric medium consisting a low amount of bile salt, pepsin, and lecithin was made by Vertzoni *et al*. (2005), which is more effectively reflecting physiological conditions of fasting state (Vertzoni *et al.*, 2005). Here, to study the antibacterial impacts of SBP, *Escherichia coli* (*E. coli)* was used as a microorganism model. *E. coli* is gram negative, rod shape bacteria that can be dangerous. It causes both harmless and critical diseases (Campbell *et al.*, 2008). This microorganism can cause widespread infection in human organs such as stomach, kidneys, intestine, and brain. It can find its way to blood and intracellular fluids, intercellular fluids, or other body fluids.

1.2 Statement of Problem

Antibiotics and antibacterial agents have been produced and applied for treatment of infectious diseases, and also they have been used in industry sector. In medical cases, these compounds have several unwanted impacts on human body that could be either harmless or dangerous.

In spite of the global spreading of SBPs as an antibacterial agent in medical, pharmacy, and industry, little researches were conducted on their effects on human body fluids. Each kind of chemical compound (e. g., drug or food) could have certain interactions with both target and non-target fluids and organs in human body. Consequently, some changes occur in SBPs framework which cause some chemical changes in human body fluids and also in their own structures.

Lots of researches about the effects of antibacterial agents on human organs as medicines have been carried out, for example the effect of silver sulfadiazine on the burned skins. It is essential for human health to find out the interactions between SBP and human body fluids or gastric fluids, and the effects on these fluids resulted from the mentioned interaction are significant.

There are not adequate researches and studies regarding these interactions and their effect. Since Body Fluid and Gastric Fluid play vital role in human body and each kind of changes in their chemical, biological, and physical features are important for human life, we conduct the current study to investigate those interactions and their effect.

1.3 Objectives of Study

The objectives of this study are listed below:

1. To prepare and characterize silver-based products**.**

2. To investigate the antibacterial activity of Silver-based products

3. To study the effect of silver based products on simulated body fluid and simulated gastric fluid

1.4 Scope of Study

The current study was conducted principally to prepare and characterize the SBPs; three kinds of silver zeolites such as silver zeolite Y, silver zeolite A, and clinoptilolite; as well as two major types of silver clay minerals: silver bentonite and silver kaolinite. Two kinds of simulated body fluids, namely, SGF and SBF were prepared in order to examine the effect of SBP in terms of their pH. Additionally, impacts of SBF and SGF on the structures of silver zeolites and silver clays were examined by Fourier Transform Infrared Spectroscopy (FTIR). Other important issue studied in this project is the antibacterial activity of SBP against *E. coli*. Antibacterial activity of each kind of silver-modified zeolites and silver-modified clays was investigated by disk diffusion technique. And antibacterial effect of SBP on contaminated SBF with *E. coli was* determined by the same technique.

1.5 Significance of Study

SBPs have been used as antibacterial agents for treating some kinds of infectious diseases. Body fluid and gastric fluid are important fluids in body that can be considered as target for SBP.

Investigation of antibacterial activity besides the harmless or dangerous effects of SBP on body fluids is crucial for human health condition. On the other hand, structural changes which were happened on SBP after being added to SBF and SGF are important from pharmaceutical and medical aspect.

SBP were added to body fluids in different concentrations. Among them, those of them that show least pH changes in the body fluids and more stability in SBP structure could be used in treating infectious body fluids with SBP in a way to have the least unwanted effect. Thus, these results can help the pharmaceutical and medical industry to produce proper medicine to treat the infectious diseases.

1.6 General Overview of the Research

Figure 1.1 illustrates the overall methodology of this study. It can be divided into three main parts. The first one is the preparation of the modified silver zeolites and silver clays and also simulated body fluid and gastric fluid, followed by the characterization of the zeolites, clays, silver zeolites and silver clays. The second part of the research includes the antibacterial assay where the antibacterial effects of each sample of silver zeolites and silver clays were determined by disk diffusion technique. The last part was about the analysis of the effects of SBPs on pH of simulated body fluids (SBF), and impacts of pH changes on SBF and SGF, and this part also discusses the structural changes of SBPs after being added to simulated body fluids in different concentrations.

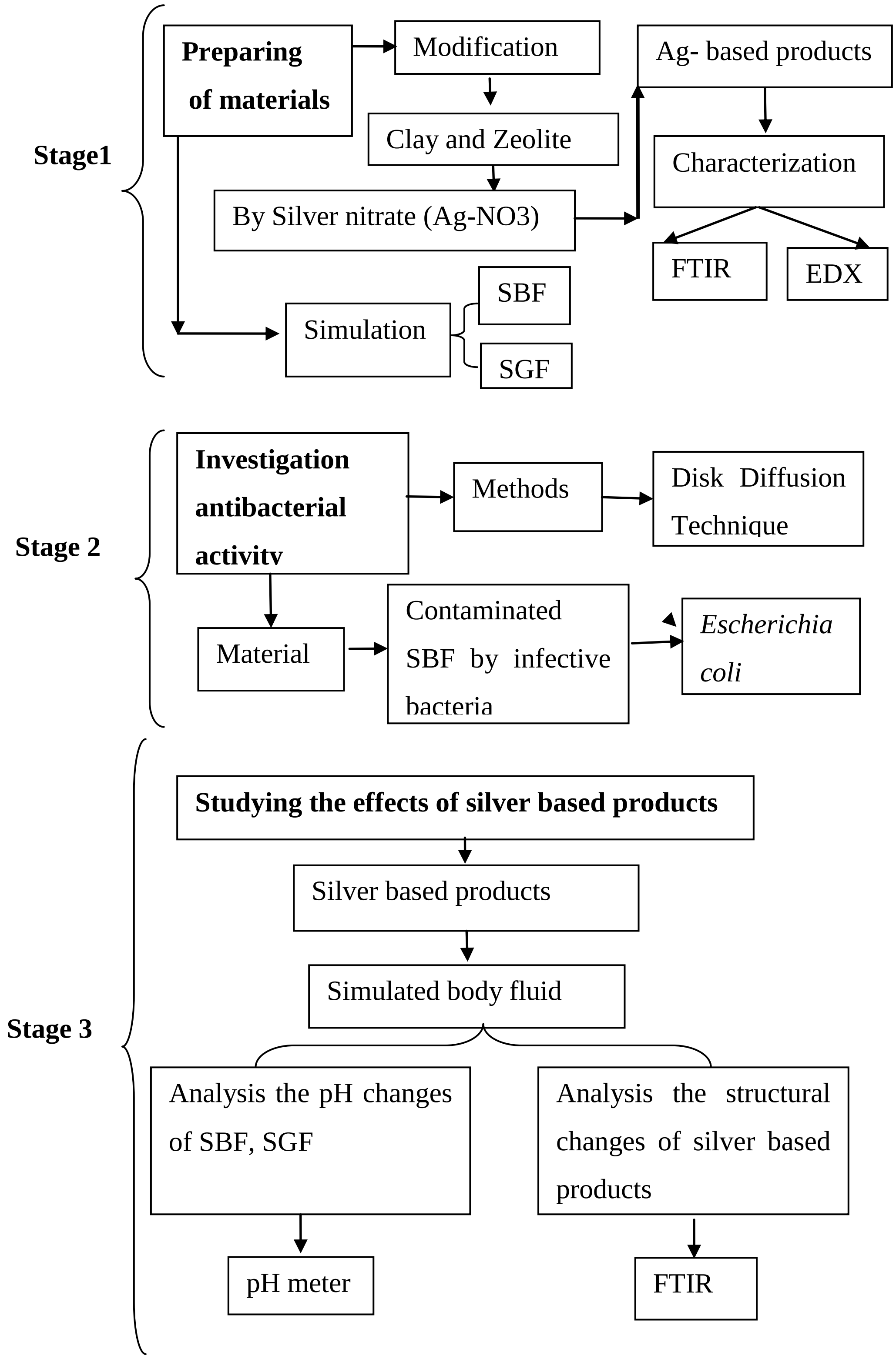

Figure 1.1 Research methodology design.

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