SYNTHESIS OF Zn(II)/SILICA BY SOL-GEL METHOD AS AN ANTIBACTERIAL MATERIAL AGAINST *Escherichia coli* AND *Staphylococcus aureus*

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ABSTRACT

Synthesis of Zn(II)/silica by sol-gel method as antibacterial material against *Escherichia coli (E.coli)* and *Staphylococcus aureus (S.aureus)* has been done. Sol-gel process was carried out by reacting tetraethoxysilane, H_2O, HCl, ethylene glycol and $ZnCl_2$. The $ZnCl_2$ concentrations varied 0.25, 0.5; 0.75, 1, and 1.25 M. Heat treatment were done by combination of microwave and oven, while the process of aging gel Zn(II)/silica carried out for 24 h and or without going through the process of aging. The Zn(II)/silica was then characterized by Fourier Transform Infrared Spectroscopy (FT-IR), X-ray diffraction method (XRD), and Transmission Electron Microscopy (TEM). The Zn(II) ions concentration that released from Zn(II)/silica was determined by AAS. Zn content in Zn(II)/silica was determined by X-Ray Fluoresence (XRF). Porosity of materials were determine by monolayer adsorption of N_2 gas. Antibacterial test was done by calculate the number of bacteria surviving after contact with Zn(II)/silica by plate count method and compared to silica.

The XRD results showed that Zn(II)/silica phase composed of amorphous SiO_2 and y-$ZnCl_2$. The porosity test results showed that Zn(II)/silica has surface area of 517.698 m^2/g. The XRF analysis results showed that $ZnCl_2$ 0.25 M produced the optimum Zn content 35,60% in Zn(II)/silica. Zn(II)/silica synthesized by heating in the oven at 150°C for 3 h after dried by microwave and aged resulted in the optimum Zn(II)/silica due to the least release of Zn(II) ions from Zn(II)/silica when analyzed by AAS (5.51%). Zn(II)/silica with concentration of 163 ppm could kill *E.coli* from concentration of 1.5 $x10^6$ CFU/mL to 0 CFU/mL in 2,5 h contact time, and reduces *S.aureus* concentration 1,2 x 10^6 CFU/mL with a contact time of 24 h. While the silica with the same concentration to that of the Zn(II)/silica showed no antibacterial effect against *E.coli* and *S.aureus.*

1. INTRODUCTION

Antibacterial materials are generally made of organic materials and inorganic materials. Inorganic antibacterial materials undergone many developments and antibacterial material is preferred because it is non-volatile, high temperature resistant and has a lower level of risk materials when compared to organic antibacterial materials (Liang et al., 2011). Inorganic antibacterial materials are made from a combination of natural or artificial mineral as a carrier of metal ions such as Ag^+, Cu^{2+}, and Zn^{2+} (Jenkins and Gilles, 2001) and have been applied as antibacterial agents in various fields such as fiber (Teli and Ravindra, 2011), textile fabrics (Singh et al., 2012), ceramics (Kim et al., 1998; Saleh et al., 2011), and teeth cleaning (Kawasaki et al., 2002).

Antibacterial properties of Ag(I) is higher than those of Cu(II) and Zn(II), but this metal ion is not essential for the organism, so that its application is limited. Zn(II) has high antibacterial properties, good stability, and relatively inexpensive (Li et al., 2002). Zinc is an essential heavy metal, though zinc is a toxic metal in high concentration. This

element is required by the body in small amounts. Toxicity of zinc will have an effect when it enters the body in large number or exceeded the tolerance (Palar, 1994). Ions Zn(II) has antibacterial effective against some Enterophatogenic bacteria such as *Escherichia coli, Staphylococcus aureus,* and *Candida albican* (Zelenak et al, 2002) also against pathogenic bacteria such as *Salmonellae, Shingellae, Vibro cholerae* (Faiz et al., 2011).

Carrier materials that can be used as a matrix-based antibacterial materials such as metal ions are silica (Trapalis et al., 2003; Rivero et al., 2011), zeolite (Zang et al., 2009), montmorillonite (Xu et al., 2011), and hidroksipatit (Kim et al., 1998). Silica is a porous material that can be used as a carrier material of metal ions, because it has good chemical stability, resistivity to acids, high pore density, heat resistance, low thermal conductivity, and does not cause pollution. Porous silica structure which causes silica have the ability to absorb metal ions that are as antibacterial, so silica is very promising for applications as an antibacterial ingredient carrier material (Matthews et al., 2010).

Appropriate method to prepare an antibacterial material that can hold and release the appropriate metal ions to produce material that can act as a slow release agent for antibacterial materials. Sol gel method is widely used in the synthesis of supported metal material. Its usefulness is based on the ease of entering one or two simultaneously active metals in the material development so that the composition of the metal material can be arranged at the time of preparation (Lambert and Gonzalez, 1998). The advantage of this method, among others, producing metal-material interactions bearers stronger so that the loss of metal during the heating process can be suppressed (Freezer and Maier, 2006), the resulting product has a high homogenity and purity (Lambert and Gonzalez, 1998), control pore size distribution in the nanometer scale, both in the form of bulk, fiber, tube, and a thin layer of particles at relatively low temperatures (Kumar et al., 2008; Nuryono and Kunarti, 2009) so that the loss of material due to evaporation can be minimized (Jamarun, 2000) . Based on these advantages in the sol-gel method, expected to be applicable to the synthesis of Zn(II)/silica which can produce material with pores and the silica content of adequate Zn and can withstand Zn and make it as slow release agents for antibacterial materials.

In the synthesis of Zn(II)/silica by sol-gel method, binding reaction takes place simultaneously with the formation of solids so that ion Zn(II) can trapped more in the silica matrix. That material is influenced by the conditions at the time of synthesis, including the concentration of reactants, heat treatment, and the aging process. The concentration of different metals give different structures and antibacterial activity (Trapalis et al., 2003). Aging and heating process to determine homogenity and pore size of Zn(II)/silica formed (Schubert and Husing, 2000). Proper warm temperatures will produce materials to suit the desired application (Sousa, 2003).

Zn(II) can be released from the Zn(II)/silica because silica act as a carrier (carrier) and slow realese (slow release) of ions Zn(II) (Ohra et al., 2005). Silica carrying ions Zn(II) has antibacterial power through the mechanism of slow release agent where the positive charge of the ions Zn(II) and a negative charge on the cell membrane of bacteria will cause attraction between ions Zn(II) and negatively charged areas in the cell walls of bacteria to form electrostatic bonds (Beveridge et al., 1989). Electrostatic bond will form stress that cause damage of cell wall permeability, reducing the normal intake of nutrients to sustain life, causing cell death.

Antibacterial activity assays of Zn(II)/silica conducted on two types of bacteria, namely *Escherichia coli (E.coli)* which is a gram-negative bacteria and *Staphylococcus aureus (S.aureus)* is a gram-positive bacteria (Jawetz, 1999). Antibacterial tests conducted using a liquid medium as a medium of bacterial growth and the number of surviving bacteria after contact with Zn(II)/silica were calculated using the plate count method (Pelczar, 2005).

Based on the description above, in this research will synthesis Zn(II)/silica by sol-gel method. Studies conducted in this research include the influence of variations of Zn(II) concentration were added to the sol silicate, examines the influence of heat treatment and aging process of the Zn(II)/silica, and antibacterial activity of Zn(II)/silica against *E.coli* and *S.aureus.*

2. MATERIALS AND METHODS

2.1. Materials and Devices

The material used in this study include Zn(II)/silica is TEOS, $ZnCl_2$, Ethylene glycol, HCl, alcohol 70%, *Escherihcia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923, nutrient agar medium (Oxoid), nutrient broth medium (Oxoid), physiological NaCl solution, and Mc Farland standards No. 1 ($3x10^8$ CFU/mL). The tools used in this study include glassware, PE containers, analytical balance (Mettler Toledo AB54-S), oven (Memmert, 100), microvawe (Sharp, Magnetron 2M167B), centrifuge bottle, vortex (Thermo Scientific), centrifuge (KOKUSAN, H-107), Atomic Absorption spectrophotometry/AAS (Perkin Elmer 5100 PC), 100-1000uL micropipette (Biohit Transferpette), autoclave, laminar air flow, vortex (Thermo Scientific). As for the material characterization Zn(II)/silica using Fourier Transform-InfraRed/FT-IR (Shimadzu Prestige-21), X-Ray Diffractometer/XRD (Multiflex, Rigaku), X-Ray Fluorescence/XRF (PAN Analytical MiniPal 4), BET (Quantachrome Nova 1200e), and Transmission Electron Microscope/TEM (JEOL, JEM-1400).

2.2. Preparation of TEOS-based silica

A total of 2.2 mL of ethylene glycol was mixed with 6.6 mL of TEOS, and then added with 1.4 mL of 37% HCl. The mixture is then stirred with a magnetic stirrer for 2 hours. Sol is heated by microwave 270 W (heating was stopped every 30 seconds) until it forms a gel, then the gel is stored in a desiccator for 24 hours. Gel was then heated to dry using a microwave (heating was stopped every 2 minutes) and then crushed. Synthesized were
characterized using FT-IR, XRD, BET and TEM.

2.3. Determination of optimum concentration of $ZnCl_2$ on the synthesis of Zn(II)/silica by sol-gel method

Composition similar to the synthesis of silica added to 10 mL of ZnCl2 0.25 M for 2 hours until homogeneous. Sol is heated by microwave 270 W (heating was stopped every 30 seconds) until it forms a gel, then stored in a desiccator for 24 hours (the aging process). Gel is then heated until dry using a microwave (heating was stopped every 2 minutes) and then crushed. The same procedure is performed for various concentration $ZnCl_2$ 0.5 M, 0.75 M, 1 M and 1.25 M. The content of Zn on Zn(II)/silica were analyzed by XRF.

2.4. Determination of the effect of different heat treatment and aging process on the synthesis of Zn(II)/silica by sol-gel method

Composition similar to the synthesis of Zn(II)/silica with $ZnCl_2$ optimum concentration, stirred for 2 hours with a magnetic stirrer until homogeneous, and then treated with the following variations:

a. Zn(II)/silica A
   Sol Zn(II)/silicate heated by microwave power 270 W to form Zn(II)/silica gel, aging for 24 h in a desiccator, and continued drying gel 270 ^oC using a microwave oven until dry, and continued drying use an oven with a temperature of 150 °C for 3 h.

b. Zn(II)/silica B
   Sol Zn(II)/silicate is heated by microwave 270 W to form a gel. Then the gel is stored in a desiccator for 24 h. Then gels were dried in an oven with a temperature of 150 °C for 3 h.
c. Zn(II)/silica C
   Sol Zn(II)/silicate is heated by an oven with a temperature of 80 °C to form a gel. Then the gel is stored in a desiccator for 24 h. Gels were then dried in an oven with a temperature of 150 °C for 3 h.
d. Zn(II)/silica D
   Sol Zn(II)/silicate is heated in an oven with a temperature of 150 °C for 3 h.

After each subsequent dry samples were measure the weighed, then crushed, and characterized by FT-IR and XRD. Test release of ions Zn(II) from Zn(II)/silica made by dissolving 0.1 grams of each Zn(II)/silica A, B, C, and D into 100 mL aquabides then shake by vortex for 3 minutes. The mixture was then centrifuged with a centrifuge at a speed of 2000 rpm for 15 minutes. Then the solution was taken and diluted 50 times and next analyzed by AAS to determine the concentration of ions Zn(II) in the filtrate.

Zn(II)/silica that release the lowest ions Zn(II) based on the results of the analysis by AAS, further characterized by IR, XRD, XRF and TEM. The specific surface area, average pore volume and pore radius determined by BET. Zn(II)/silica optimum is then used to test antibacterial activity against *E.coli* and *S.aureus* and compared with silica.

2.5. Antibacterial test Zn(II)/silica against *E.coli*

Suspensions of Zn(II)/silica prepared by dissolving as much as 32.6 mg Zn(II)/silica into 100 mL sterile aquabides then shake by the vortex. Silica suspension was prepared by dissolving 32.6 mg of silica in 100 mL into sterile aquabides then shake by the vortex. A total of 25 mL suspension of *E.coli* cells (1.5×10^6 CFU / mL) was added 25 mL solution of Zn(II)/silica, then shake for variation within 30, 60, 90, 120, 150, 180 min and 24 h, and then calculated the number of surviving bacteria using plate count method. The same procedure is done by adding a silica suspension into a suspension of *E.coli* to compare the antibacterial properties against *E.coli.*

2.6. Antibacterial test Zn(II)/silica against *S.aureus*

The same procedure as 5 is performed by adding a suspension of Zn(II)/silica into a suspension of *S.aureus* and suspension silica into the suspension *S.aureus,* then compared the antibacterial properties.

3. RESULTS AND DISCUSSION

3.1. Characterization of the structure of silica and Zn(II)/silica by FT-IR

FT-IR spectra in Figure 1 shows that the silica and Zn(II)/silica has a characteristic infrared absorption patterns that are relatively similar. Absorption at wavenumber 455.20 cm^{-1} for silica and at 462.92 cm^{-1} for Zn(II)/silica that show bending vibration absorption =Si-O of =Si-O-Si= (Innocenzi, 2003; Wogo, et.al, 2011). Absorption at wavenumber 794.67 cm^{-1} in silica and Zn(II)/silica showed symmetric stretching vibrations of =Si-O of =Si-O-Si= (Innocenzifrom 2003). Absorption at wave numbers 1087.85 cm^{-1} for silica and 1080.14 cm^{-1} for Zn(II)/silica showed asymmetric stretching vibration absorption =Si-O of =Si-O-Si= (Beganskiene et al., 2004 ; Nuryono and Narsito, 2005). Wavenumber spectra at 964.41 cm^{-1} for silica and Zn(II)/silica showed asymmetric stretching vibration absorption =Si-O of =Si-OH. Wide absorption band at wavenumber 3387.00 cm^{-1} on silica and wave number 3448.72 cm^{-1} on Zn(II)/silica showed symmetric stretching vibration absorption -OH from =Si-OH or vibrations of

hydrated water (Silverstein, et.al., 1981). Wavenumber spectra at 1643.35 cm^{-1} on silica and wave number 1627.92 cm^{-1} on Zn(II)/silica which is the H-O-H deformation that interact through hydrogen bonds with silanol groups (=Si-OH) (Beganskiene et al., 2004).

In the FTIR spectra in the visible presence of vibration absorption =Si-O bending, symmetric stretching vibrations of =Si-O and asymmetric stretching vibrations of =Si-O from wavenumber shift characteristics of =Si-O-Si= (siloxane) on Zn(II)/silica toward smaller wavenumbers of the absorption on silica. Wavenumber shift toward smaller indicate an interaction between the ions Zn(II) to the oxygen atom of the Si-O groups, leading to weakening of the Si-O bonds (Buhani et.al., 2011).

The estimated structural models of silica and Zn(II)/silica formed is shown in Figure 2. Figure 2(a) shows the silica monomer composed of siloxane (Si-O-Si) and silanol (Si-OH). Figure 2(b) shows the model structure of Zn(II)/silica formed, where the metal Zn(II) occupies the silica matrix or

framework. Interaction between Zn(II) with silica matrix possibly through the formation of complexes in which Zn(II) plays as a central atom and silica matrix as a ligand.

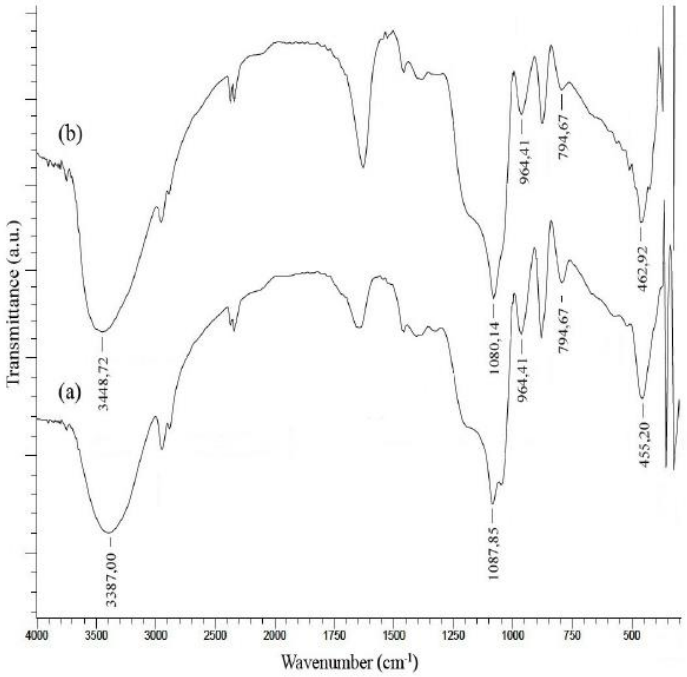

Fig. 1. FTIR spectra of (a) silica and (b) Zn(II)/silica

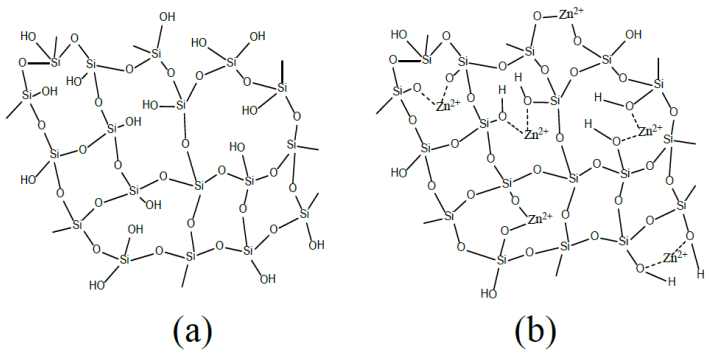

Fig. 2. Structure model of (a) silica and (b) Zn(II)/silica

3.2. Characterization of silica and Zn(II)/silica by X-ray diffraction method

Characterization of silica and Zn(II)/silica using X-ray diffraction was conducted to identify the structure of silica and Zn(II)/silica concentrations of $ZnCl_2$ crystallography. Figure 3(a) shows amorphous SiO_2 phase diffraction pattern of silica synthesized which can be determined by the appearance of diffraction peaks at 21.90° (d=4.055A) and 23.8° (d=3.735A). Reflection peak is spacing between molecular states in SiO_2, widening the angle of diffraction peaks reflecting 19°-24° gives an indication of the arrangement of Si and O are random and produces amorphous structure. Based on the diffraction pattern of Zn(II)/silica that are presented in Figure 3(b), X-ray diffraction pattern of Zn(II)/silica synthesis produces a diffraction peak widening of the diffractogram silica, this is due to the interaction of Zn with SiO_2 thus distorting Si-O bonds in silica. Distortions caused a marked decrease of crystallinity SiO_2 with SiO_2 widening reflection. Zn in Zn(II)/silica present in the form of y-$ZnCl_2$ known through matching with the data JCPDS No.18-0850. Characteristics of y-$ZnCl_2$ can be observed at the summit include the diffraction angle 26.14° (d=4.406A), 28.88° (d=3.089), and 47.92° (d=1.896).

3.3. Content analysis of Zn in the Zn(II)/silica

Figure 4 shows the results of the analysis of the levels of Si and Zn in Zn(II)/silica variations in the concentration of $ZnCl_2$, where the decline in the levels of Si as well as increased levels of Zn in the Zn(II)/silica with increasing concentrations of $ZnCl_2$ were added to the silicate sol. Obtained the highest Zn content of Zn(II)/silica at 1.25 M $ZnCl_2$ that is equal to 50.14%, but Si contained very low at 8.40%. Very low content of Si is not sufficient as the main constituent elements of Zn metal carrier is silica. Zn(II)/ silica concentration of 0.25 M $ZnCl_2$ contains Si adequate than others, namely by 18% and amounted 35.60% for Zn content, and then used as the concentration of $ZnCl_2$ for the synthesis of Zn(II)/silica variations in heat treatment and gelation process.

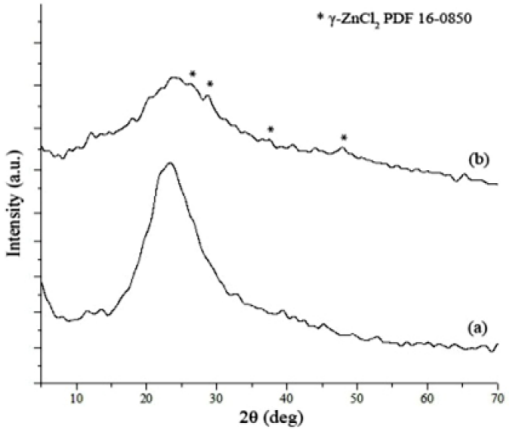

Fig. 3. X-ray diffractogram (a) silica and (b) Zn(II)/silica

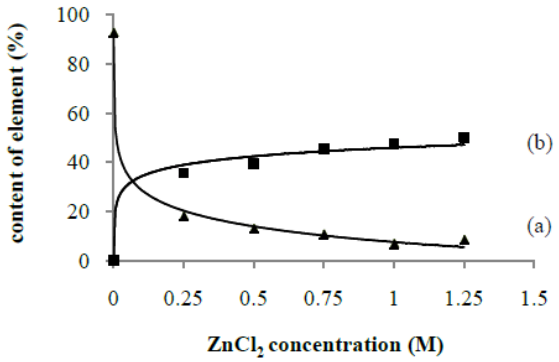

Fig. 4. XRF analysis results for (a) silica and (b) Zn(II)/silica

3.4. Characterization of Zn(II)/silica A, B, C and D with FT-IR

FTIR spectra in Figure 5 show that Zn(II)/silica A, B, C and D provide similar absorption characteristics. Absorption characteristics of the silica, Zn(II)/silica A, B, C and D are shown in Table 1. Figure 5 shows the FTIR absorption of silica and Zn(II)/silica variations in heat treatment and aging process of Zn(II)/silica A, B, C, and D. Variations in heat treatment and aging process does not make a difference absorption symmetric and asymmetric stretching vibrations of =Si-O at 794.67 cm^{-1} and 1080.14 cm^{-1}. Zn(II)/silica method A, B, and C produce the same vibration absorption of =Si-O at 455.20 cm^{-1} while the bending vibration absorption of =Si-O for Zn(II)/silica method D at 462.92 cm^{-1}. Heat treatment during the process of gelation, aging, and drying of gel Zn(II)/silica method D were performed using the oven at 150^{o}C for 3 hours in the absence of the aging process, causing wavenumber for bending vibrations of =Si-O shift towards a more large and increase energy so that the interaction stability of Si-O bond become weak. Stretching vibration -OH for Zn(II)/silica method A, B and D, ie at 3448.72 cm^{-1}, while for method C at larger wave numbers is 3464.15 cm^{-1}.

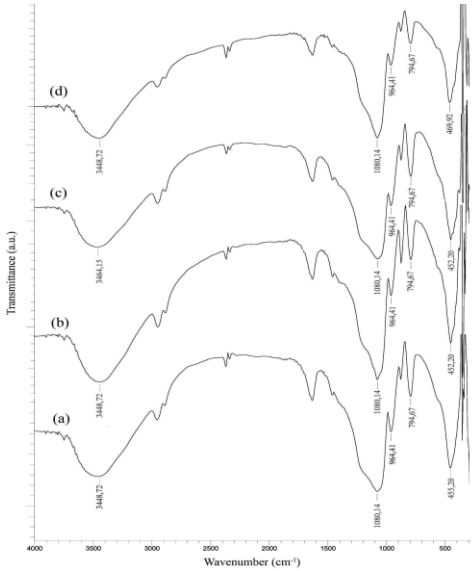

Fig.5. FTIR spectra of Zn(II)/silica (a) A, (b) B, (c) C and (d) D

Table 1. Absorption characteristics of the Zn(II)/silica A, B, C and D

Characteristic Absorption	Wavenumber (cm^{-1})				
	Silica	Zn(II)/silica			
		A	B	C	D
Bending vibration of =Si-O from (=Si-O-Si=)	455,20	455,20	455,20	455,20	462,92
Symmetris stretching vibration of =Si-O from (=Si-O-Si=)	794,67	794,67	794,67	794,67	794,67
Stretching vibration of =Si-O from =Si-OH	964,41	964,41	964,41	964,41	964,41
Asymmetris stretching vibration of =Si-O from (=Si-O-Si=)	1087,85	1080,14	1080,14	1080,14	1080,14
Stretching vibration of -OH from =Si-OH	3387,00	3448,72	3448,72	3464,15	3448,72

3.5. Characterization of Zn(II)/silica A, B, C and D with X-ray diffraction method

Diffractogram of Zn(II)/silica A, B, C and D in Figure 6. Table 2 shows the Zn(II)/silica has two crystalline phases, namely SiO_2 tridymite phases correspond to the reference PDF 18-1169 and y-$ZnCl_2$ with PDF reference 16-0850. The presence of y-$ZnCl_2$ phase indicate that soluble metal mixed with silicate sol to form a polymer network $ZnCl_2$/silikat salt and given heat treatment does not eliminate all salt from the metal phase. The difference in treatment of the synthesis method on the four samples showed no significant difference in the diffraction pattern, making it difficult to claim the difference.

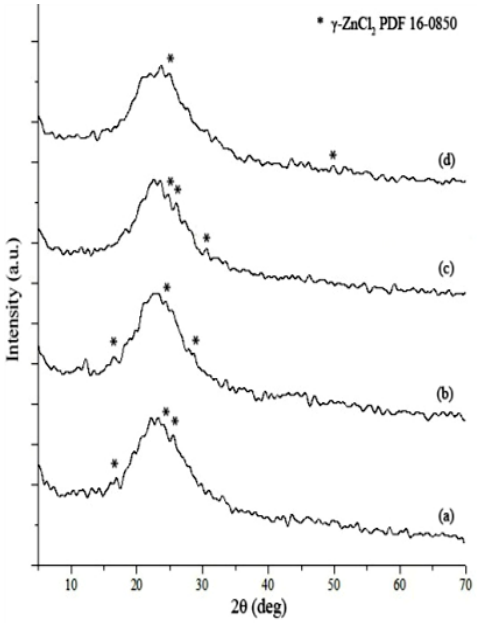

Fig. 6. X-ray diffractogram of Zn(II)/silica (a) A, (b) B, (c) C and (d) D

Table 2. Characteristics peak of ZnCl2 on Zn(II)/silica A, B, C and D compared with γ-ZnCl2 JCPDS data No.16-0850

Zn(II)/silica	*2θ*	Relative Intensity (%)	d spacing (Å)
A	16,12	81,8	5,493
	22,34	90,9	3,976
	23,24	100	3,824
	25,42	72,7	3,501
	26,34	90,9	3,381
B	16,22	100	5,459
	21,12	62,5	4,203
	23,38	87,5	3,802
	24,66	100	3,607
	28,64	62,5	3,114
C	21,66	54,5	4,099
	23,70	72,7	3,751
	24,70	72,7	3,601
	25,62	100	3,474
	30,38	54,5	2,939
D	21,08	80	4,211
	23,88	100	3,723
	24,68	80	3,604
	49,74	70	1,832

3.6. Determination of the concentration of ions Zn(II) is separated from the Zn(II)/silica A, B, C and D with AAS

The results of metal ion release test of Zn(II) from Zn(II)/silica A, B, C, and D in the medium of water respectively are 5.51%, 6.61%, 6.06%, and 6.17%. Zn(II)/silica A release Zn lower than the Zn(II)/silica that were synthesized using B, C, and D methods, which amounted to 5.51%. Percentage 5.51% of Zn were release shows the number of Zn(II) that bound physically in silica matrix, so it is easily hydrated in water. In the process of Zn(II)/silica with A method, Zn-silicate sol was heated in a microwave to form a gel and then undergo a process of aging for 1 night, dried with microwave and heating resumed for 3 hours at a temperature of 150 ^oC using the oven, so that Zn(II) are more tightly bound in a matrix of silica and become harden Zn(II)/silica materials, resulting ions Zn(II) that is released from the Zn(II)/silica A lower than the Zn(II)/silica synthesized by B, C and D method.

The determination of Zn(II) concentration that is released from the Zn(II)/silica with AAS, used Zn(II)/silica A to test antibacterial activity against *E.coli* and *S.aureus*, because the percentage of loosing ions Zn(II) is low so hopefully Zn(II)/silica is able to work on the principle of slow release when contact with bacteria so can inhibit and

cause death in bacteria. Zn(II)/silica is then characterized by TEM and XRF were compared with silica.

3.7. Characterization of Zn(II)/silica and silica with BET

Data in Table 3 shows the specific surface area silica decrease 87.015 m^2/g from 604.713 m^2/g to 517.698 m^2/g after the presence of Zn(II), indicating that $ZnCl_2$ dispersed on the surface of silica and occupy the porous that caused the specific surface area of Zn(II)/silica relatively smaller than the specific surface area of silica. Pore volume of silica increased by 0.043 cm^3/g after the presence of ions Zn(II), from 0.223 cm^3/g to 0.254 cm^3/g, which indicates $ZnCl_2$ covering the surface of the pores of the silica or silica into the pore. The presence of Zn(II) on the silica matrix also decreases the average silica pore radius of 0.23 A from 16.805 A to 16.689 A. Interaction between Zn(II) with silica matrix possibly through complex formation as illustrated in Figure 2(b), where the Zn(II) plays as a central atom and silica matrix as a ligand. The formation of this complex causes silica pores radius of the matrix decreases.

Table 3. Comparison of specific surface area, average pore volume and pore faint on silica and Zn(II)/silica

Material	Spesific SurfaceArea (m_2/g)	Pore Radius (Å)	Pore Volume (cc/g)
Silica	604,713	16,805	0,223
Zn(II)/silica	517,698	16,689	0,254

3.8. Characterization of silica and Zn(II)/silica by TEM

Based on the TEM picture in Figure 7(a) it can be seen that the morphology of the formed silica is aglomeration particulates with a relatively uniform size and relatively arranged. Nevertheless can not be determined accurately the size of the formed silica particles.

TEM picture in Figure 7(b) shows that the morphology of the Zn(II)/silica distributed irregularly, which saw the metal phase Zn(II) were deposited on the silica surface and resembles a sheet-like or plate-like. This is confirmed by the appearance of peaks $ZnCl_2$ on the X-ray diffractogram of Zn(II)/silica and that was supported by analysis using the BET surface area, where the surface area of Zn(II)/silica smaller than silica as a silica surface covered by $ZnCl_2$.

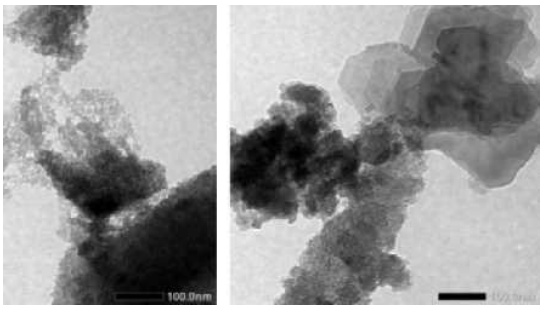

Fig. 7. TEM analysis results (a) silica and (b) Zn(II)/silic

3.9. Analysis of elemental content in the silica and Zn(II)/silica with XRF

From the analysis of elemental content by XRF, it is known that the decrease of Si in the Zn(II)/silica is from 98.90% to 58.20% and in the presence of Zn at 40.68%. The amount of Zn at 40.68% in Zn(II)/silica sol-gel process explains Zn in silica relative success and then tested for antibacterial activity against *E.coli* and *S.aureus.*

3.10. Antibacterial Test of Zn(II)/silica against *E.coli*

Minimum inhibitory concentration for ions Zn(II) is equal to 1 mM or 65 ppm (Spain and Alm, 2003; Nies, 1999). Based on the results of XRF analysis, the content of Zn in Zn (II)/silica is 40.68% (w/w), so Zn(II)/silica 163 ppm that used in antibacterial test against *E.coli* and *S.aureus* contain 65 ppm Zn(II) (1 mM). In Figure 8 can be seen that after contacted with Zn(II)/silica, the number of *E.coli* cells decrease with increasing contact time. *E.coli* concentrations were initially 1.5 × 10 CFU/mL became 0 CFU/mL after contact time for 150 minutes, whereas when contacted with the silica suspension of the same concentration, the concentration of bacteria was not reduced, but tended to increase or remain grow normally, 1.5×10^6 CFU/mL to 6.7×10^6 CFU/mL at 24 hours contact time. This suggests that the silica was not able to be antibacterial against *E.coli.*

3.11. Antibacterial test of Zn(II)/silica against *S.aureus*

In Figure 9, it can be seen that the Zn(II)/silica 163 ppm can reduce the growth of *S.aureus* bacteria at 1.2 x 10^6 CFU/mL of initial concentration of 1.5×10^6 CFU/mL to 3×10^5 CFU/mL at the time of 24 contact hours and incubation for 24 hours. Although Zn(II)/silica 163 ppm showed antibacterial activity against *S.aureus,* but not enough to effectively kill the bacteria *S.aureus* with a concentration of 1.5×10^6 CFU/mL. While the influence of silica against *S.aureus* is likely to increase from the initial concentration of 1.5×10^6 CFU/mL to 5.7×10^6 CFU/mL at 24 hours contact time, which indicates silica is not antibacterial against *S.aureus.*

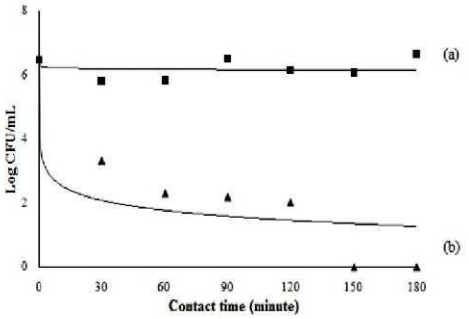

Fig. 8. The number of *E.coli* cells after contact with (a) silica and (b) Zn(II)/silica

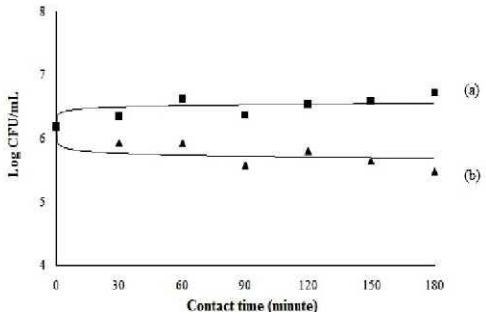

Fig. 9 The number of *S.aureus* bacteria after contact with (a) silica and (b) Zn(II)/silica

4. CONCLUSIONS

Zn(II)/silica has been synthesized by sol-gel method with 0.25 M $ZnCl_2$ that give the optimum content of Zn in the Zn(II)/silica amounted to 35.60% (w/w). XRD results indicate that Zn(II)/silica is composed of amorphous SiO_2 and y-$ZnCl_2$ phase. BET characterization results showed that Zn(II)/silica has a surface area of 517.698 m^2/g while the silica has a surface area of 604.713 m^2/g. Zn (II)/silica that is synthesized

through gelation process using microwave heating, followed by a 24 hour aging gel process, and gel drying oven for 3 hours in 150^{o}C, was the optimum condition of Zn(II)/silica because it shows the lowest concentration of Zn(II) that can release from Zn(II)/silica when analyzed by AAS is equal to 5.51%. Zn(II)/silica 163 ppm was able to kill *E.coli* bacteria from concentrations of 1.5×10^6 CFU/mL to 0 CFU/mL with a contact time of 2.5 hours. Zn(II)/silica was only able to kill *S.aureus* colonies of 1.2×10^6 CFU/mL with a contact time of 24 hours. Silica concentration of 163 ppm didn't showed antibacterial effect against *E.coli* and *S.aureus.*

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