Impregnation of silver in zeolite-chitosan composite: Thermal stability and Sterility Study


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Running head: Impregnation of silver in zeolite-chitosan composite

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ABSTRACT: The solvent-casting method was used to synthesize a silver-zeolite-chitosan (AgZ-Ch) composite. Characterization techniques such as X-ray diffraction (XRD), ultraviolet-visible (UV-Vis) spectroscopy, optical emission spectroscopy (OES), thermogravimetric analysis (TGA), and differential thermogravimetry (DTG) were used to investigate the different properties of the composite before and after plasma treatment. XRD pattern showed that majority of the phase composition of the Philippine natural zeolite is mostly composed of Na-clinoptilolite with trace amounts
of mordenite, feldspar, and quartz. UV-Vis and OES analyses confirmed the presence of Ag and zeolite on the chitosan matrix where a decrease in the transmittance peak at 290 nm and the emission spectra of the discharge showed the presence of Ag I, Al I and Si I peaks at 705-852 nm were observed, respectively. TGA and DTG curves revealed the thermal stability of the natural zeolites after ion-exchange and after its incorporation in the chitosan matrix where the onset of degradation was observed to be more than the human body temperature of around 37°C. Bacterial count showed minimal growth of colonies to all samples whether pristine or plasma-treated. This implies that the surface of the composites does not influence bacterial habitation. With these results, it is observed that the fabricated composites have met the minimum requirement for biomedical application such as thermal stability with respect to average human body temperature and bacteria-free.

Keywords: surface interactions, silver, zeolites, chitosan, plasma, degradation, transmittance.
INTRODUCTION

Aluminosilicate minerals have already existed in traditional medicine and pharmaceutical industries (Ghadiri, Chrzanowski & Rohanizadeh, 2015) (Boniferri et al. 2007). Specifically, clay minerals are one of the oldest earth materials employed for traditional healing purposes since indigenous people around the world primarily uses these externally in baths to cure skin diseases and simple gastrointestinal ailments such as diarrhea (Ghadiri, Chrzanowski & Rohanizadeh, 2015). These phyllosilicates, also known as sheet silicate minerals containing hydrated aluminosilicates arranged in either tetrahedral or octahedral sheets, are continuously used due to the many advantages of clay minerals on modern-day applications. Clay minerals have high adsorption and cation exchange capacity (CEC), colloidal and swelling capacity, optimal rheological behavior, and high water dispersibility. These physicochemical characteristics are identified to be essential in a wide range of biological applications such as veterinary medicine, pharmaceutics, cosmetics, biomaterials and biosensors (Ghadiri, Chrzanowski & Rohanizadeh, 2015). Moreover, clay minerals may generally serve as carrier, binder, or diluent agents for these industrially significant applications (Cerri, de’ Gennaro et al, 2004).

Aside from clays, zeolites are also considered to be among the most abundant natural resources in the world. Zeolites are similar to clays such that the former is also composed of aluminosilicates described as microporous molecular sieves with silicon (Si)-aluminum (Al) tetrahedral framework structures linked by bridges of oxygen (O)
between them (Ferreira et al. 2012). The zeolite structure has interconnected voids and channels which are being occupied by native cations. With this, cation exchange sites of zeolites occur within the voids and channels where the distribution and presence of native cations allow smooth cation exchange when immersed in a solution filled with other exchangeable cations (Ferreira et al. 2012). This cation exchange mechanism of zeolites, aside from the similarity in the aluminosilicate structure, is observed to be the same with clay minerals (Bonferoni et al. 2007) (Cerri et al. 2004). Adsorption and reversible dehydration are also some of the significant properties shared by zeolites and clays which make them indispensable in the industrial and biological applications (Cerri et al. 2016).

Research on zeolites for biological applications has increasingly becoming an area of interest. Studies on biomedical use of zeolites are relatively new compared to studies on clays. In fact, the quality of clays for biological applications has been established by several studies. For instance, pharmaceutical use of clays has been specified in Pharmacopeia standards (Dardir et al. 2018) (Carretero & Pozo, 2009) (European Pharmacopeia 2005) (US Pharmacopeia 2007) (Alkrad et al. 2017) (Tomasevic-Canovic, 2005). It is projected that the utilization of zeolites, specifically the Philippine natural zeolites, will be maximized in the future due to the features they possess which include a well-defined structure, ability to reversely bind to small molecules, shape and size selectivity, and metallo-enzyme behavior and immune system regulator property (Cerri et al. 2016) (Demirci et al. 2013) (Inoue et al. 2002). Zeolites can act as inorganic reservoirs for hosting ions such as zinc (Zn) (Olegario et
al. 2019) (Olegario-Sanchez & Pelicano, 2017), copper (Cu) (Montallana, Cruz & Vasquez Jr., 2018) and silver (Ag) (Osonio & Vasquez Jr., 2018) to provide prevention on possible concentration-dependent toxicity as well as to control and regulate ionic release for antibacterial applications (Ferreira et al. 2012). The rigid and stable structure of zeolites allowed suitable function in nanotechnology, heterogeneous catalysis, ion exchangers and sorbents (Ferreira et al. 2012). In addition, zeolites are considered promising carriers for drug delivery systems because of their excellent adsorption and cation exchange capabilities. These minerals can interact with different molecules and, with their porous structure, can transfer atoms or molecules from their crystals to the environment and vice versa (Barbosa et al. 2016) (Taaca & Vasquez Jr., 2017).

Zeolites dispersed as fillers in a polymer matrix are already being explored in the biomedical applications (Taaca & Vasquez Jr., 2018) (Taaca, Olegario & Vasquez Jr., 2018) (Barbosa et al. 2016) (Ciobanu, Carja, & Ciobanu, 2007) (Namekawa et al. 2014) (Ramasubramanian et al. 2015). Several zeolite-based composites have already been fabricated using different techniques such as extrusion, melt-mixing compounding, freeze-drying, precipitation and crosslinking. These various zeolite-based composites include hydroxyapatite/zeolite-ZSM5 (ZSM-HA) (Iqbal et al. 2016), titanium/ MFI zeolite (M-Ti) (Li et al. 2015), and gelatin/zeolite (Ninan et al. 2013) where noteworthy results were observed. The bioactivity of ZSM-HA was detected when normal human osteoblasts adhered and proliferated on the surface of the composites (Iqbal et al. 2016). Ti alloys coated with MFI zeolites were observed to
have enhanced osteointegration and promoted bone regeneration when implanted in rabbit femoral condylar defects at 4 and 12 weeks (Li et al. 2015). The porosity, swelling, and degradation properties of the zeolite-gelatin scaffold showed potential towards regenerative medicine and tissue engineering (Ninan et al. 2013).

In our previous works, a composite made from Ag-zeolite (AgZ) and chitosan (Ch) was successfully fabricated using a solvent casting approach and evaluated its antibacterial, cytocompatibility, and hemocompatibility properties for possible biomedical applications (Taaca and Vasquez, 2017) (Taaca and Vasquez, 2018). There are already existing chitosan-zeolite studies for biomedical application (Zhang et al. 2015) (Yu et al. 2013). However, surface modification via plasma treatment was not yet developed to tailor the surface properties of the composite. Plasma modification was also utilized in our works as this treatment is considered to be an effective method in enhancing the wettability and surface roughness of polymers, such as chitosan, and ceramic materials, like zeolites (Cagomoc and Vasquez Jr., 2016) (Osonio and Vasquez, 2018). Previously, the porosity and the surface properties (roughness and wettability) of chitosan were greatly enhanced with the presence of AgZ particles coupled with plasma treatment, respectively (Taaca and Vasquez, 2017). It was also revealed that the AgZ fillers enhanced not only the antibacterial activity of chitosan but also its blood clotting ability without promoting adverse effects (Taaca and Vasquez Jr., 2018).

In this study, composites of AgZ-Ch were synthesized and its thermal response was evaluated. In addition, presence of microorganisms in the composite surface was
investigated. Locally-sourced natural zeolites were characterized through X-ray diffraction (XRD) technique. The properties of the composites were determined using thermogravimetric analysis (TGA) and ultraviolet-visible (UV-Vis) spectroscopy. The gas discharge was analyzed using an optical emission spectrometer (OES).

MATERIALS

The zeolite samples used as supplied by SAILE Industries, Inc., were mined in the town of Mangatarem, province of Pangasinan, Philippines. The natural zeolite samples underwent pretreatment to produce sodium-exchanged zeolite (NaZ). Medium-molecular weight (44487-50G) chitosan was purchased from Sigma-Aldrich. Analytical grade acetic acid (CH₃COOH), from Macron, locally-sourced 99% silver nitrate (AgNO₃), glycerine and isopropyl alcohol were also procured for the synthesis of the composites. All materials were used as received unless specifically mentioned in the succeeding steps. Deionized water (DI H₂O) was used all throughout the experimental runs.

EXPERIMENTAL

Ion-exchange Method
The ion-exchange process used in this study is similar to the methods done in refs. (Taaca & Vasquez Jr., 2017) (Taaca & Vasquez Jr., 2018). The as-received NaZ minerals were powdered using mortar and pestle and washed with 100 mL DI H₂O. Filtration was done twice after each washing of the powders. Drying and active treatment of the powders followed at temperatures 105°C and 120°C for 2 h and 6 h, respectively. The ion exchange process started by soaking the actively-treated NaZ powders in a 0.05 M AgNO₃ solution at room temperature (RT) overnight, with a 1:20 ratio of NaZ powder and AgNO₃ solution. The mixture was then stirred rigorously for an additional 12 h to continue the ion exchange process at RT. The AgZ product was obtained through washing and filtering of the mixture. These steps were done twice after the ion exchange process. The filtered Ag-exchanged zeolites (AgZ) were then dried at 105°C for 2 h. The final step of the ion exchange process was calcination where it was done at 400°C for 5 h. The calcination step reduces the Ag⁺ ions, impregnated into the zeolite, to metallic Ag. Metallic Ag is known to be more effective as an antibacterial agent due to the regulated release of Ag⁺ ions. (Xiu et al. 2012) (Osonio and Vasquez, 2018)

Synthesis of AgZ-Ch composites

The Ag-exchanged zeolite/chitosan (AgZ-Ch) composites were prepared by mixing the chitosan and the zeolite suspensions via solvent casting method for 2 h (Taaca and Vasquez Jr., 2017). The chitosan suspension was composed of 2 wt% Ch
and 90% CH₃COOH solution stirred at 380 rpm and temperature of 60°C. The AgZ suspension was done by dispersing 5% (w/v) AgZ in DI H₂O sonicated for half an hour. Different AgZ-Ch mixtures were prepared in this study by adding varying contents of zeolites ranging from 0 to 2.0 wt% and mixing for another 1 h. Glycerine was also added as plasticizer to each of the AgZ-Ch mixture. The mixtures were then casted on a polystyrene petri dish and air-dried for 2 to 3 days. Dried AgZ-Ch sheets were soaked with isopropyl alcohol for 1 h as post-treatment. The final dried samples were sealed in a desiccator prior to plasma treatment.

Plasma Treatment of AgZ-Ch Composites

The plasma setup used in this study is similar to the system used in the previous studies of the locally-sourced natural zeolites (Cagomoc & Vasquez Jr., 2016) (Osonio & Vasquez Jr., 2018) (Taaca & Vasquez Jr., 2017) (Taaca & Vasquez Jr., 2018). The plasma system utilizes a 13.56 MHz radio frequency (RF) power with a manually-tuned matching network. For all runs, 99.99% argon (Ar) gas was used. Prior to treatment, the system was plasma-cleaned to remove possible gas impurities present inside the chamber.

Selected dried sheets of 0% AgZ-Ch composites (0% AgZ-Ch and 1.0% AgZ-Ch) were exposed to Ar discharge plasma after chamber cleaning. This was done to determine the effect of plasma discharge to the composites, with or without AgZ. The plasma system was first evacuated to base pressures below 10 Pa. The experiments
were conducted under an incident RF power of 50 W and pressures 16 Pa and 30 Pa for
2 min. Low pressure was used in this study to modify the surface properties of the
AgZ-Ch composites without significantly affecting the bulk properties.

Characterization

The zeolite phase composition of the as-received NaZ sample was determined
using X-ray diffraction (XRD) analysis, Shimadzu Maxima XRD-7000, with a scan
speed of 0.02°/min ranging from 2° to 70°. The NaZ powder samples were pressed on
a double-sided tape and mounted on a glass slide prior to characterization. The
composites were evaluated using a PANalytical X’Pert system with a continuous scan
mode and range of 5°-80°. Thermogravimetric analysis (TGA) was used to evaluate
the thermal stability of AgZ and AgZ-Ch composites. The analyses of AgZ and AgZ-Ch
composites were conducted using TGA Q500 under a dynamic N₂ atmosphere
(100mL/min) from 30°-700°C at a rate of 10°C/min. Ultraviolet-visible (UV-Vis)
absorption spectra of the AgZ-Ch composites were measured using Shimadzu UV mini
1240 spectrometer. The baseline of the UV-Vis analysis was corrected to 200 nm with
a scanning range of 0-1200 nm. Optical emission spectroscopy (OES) was used to
identify the ionic species detected in the plasma discharges during the plasma
treatment of the composites at different working pressures. The fiber optic detector is
placed at the plasma system and in line with the bulk plasma at the center of the glass
chamber. The detector is connected to a Horiba iHR320 spectrometer. Analysis of the
bulk plasma was done by identifying the spectral lines using Spectral Analyzer 1.7 software (Navratil et al. 2006). The spectrum of the discharge in each step was observed for any changes throughout the process. Bacteria count of the pristine and selected plasma-treated AgZ-Ch composites was done under the services of the Microbiological Research and Services Laboratory (MRSL) of the Natural Sciences Research Institute (NSRI) in UP Diliman. The composites were washed with 9 mL 0.1% peptone water for 2 min. The washings were diluted up to $10^2$. Dilutions were pour-plated in Plate Count Agar (PCA) in duplicates. Plates were incubated at 35°C for 48 h before counting colonies under a Quebec colony counter. The weighted mean count (WMC) is expressed as colony forming units (CFU) per gram sample and is calculated using the formula (Eq. 1):

$$WMC = \frac{n}{(f_a \times 1) + (f_b \times 0.1) + (f_c \times 0.01) + \ldots} \times df$$  \hspace{1cm} (Eq. 1)

Where $n$ represents the total number of colonies in all plates counted, $f_a$ is the number of plates with the first countable dilution, $f_b$ and $f_c$ is the number of plates in the succeeding dilutions, and $df$ is the dilution factor, which is also equal to the reciprocal of $f_a$. For all characterizations, 1cm x 1cm dried sheets of AgZ-Ch composites and 3µg of zeolite were used.

RESULTS AND DISCUSSION
XRD of as-received NaZ and the AgZ-Ch composites

Fig. 1 X-ray diffractogram of the as-received NaZ sample. Characteristic peaks correspond to Na-clinoptilolite (CLI), mordenite (MOR), feldspar (F) and quartz (Q).

The XRD spectrum of the as-received NaZ sample is shown in Fig. 1. Analysis revealed that the natural zeolite used in this study is composed of Na-clinoptilolite (Alberti, 1975), mordenite (Martucci et al. 2003), feldspar (Grundy & Ito, 1974) and quartz (Levien et al. 1980), respectively. The Na-clinoptilolite peaks are identified at 2θ angles 9.8°, 11.32°, 13.33°, 15.08°, 17.38°, 19.58°, 22.12°, 23.52°, 25.65°, 26.26°, 27.92°, 30.9° and 35.62°. Moreover, characteristic peaks of mordenite, a trace zeolitic mineral, were also identified within the 3° to 40° scan range. The presence of other
zeolite peaks is possible since no purification was done to the natural zeolite samples prior to ion-exchange of Na$^+$ ions. Traces of other mineral impurities such as feldspar and quartz were also identified in the diffractogram.

Figure 2 shows the XRD patterns for the pristine AgZ-Ch composites. Chitosan has a broad characteristic peak around the 20-25 angles, centered at approximately $2\theta = 21^\circ$ (Taaca and Vasquez Jr., 2018). Based from the graph, the characteristic peak of chitosan did not drastically change upon the addition of AgZ particles.

![XRD pattern of the pristine AgZ-Ch composites with varying AgZ contents (0.5% to 2.0%)](image)

**Fig. 2** XRD pattern of the pristine AgZ-Ch composites with varying AgZ contents (0.5% to 2.0%)

**UV-Vis Analyses of the AgZ-Ch composite samples**
UV-Vis spectrometry was used to detect the presence of AgZ in the AgZ-Ch composites. The pure chitosan, 0% AgZ-Ch composite, has characteristic peaks at 240 nm and 290 nm, as shown in Fig. 3. These peaks can be attributed to the glucosamine unit of chitosan and the chemical interaction of CH$_3$COOH and chitosan at RT, respectively. Decreasing intensities of the transmittance peak at 290 nm are observed after incorporating 0.5 to 2.0 wt% of AgZ powders into the chitosan matrix. The intensity values changed from a value of 79.32 up to a range of 1.06 to 68.7. In addition, the decreasing trend of the transmittance with increasing AgZ content indicates that AgZ particles in the matrix causes the increase in the UV absorption
where the 2.0% AgZ-Ch composite has the lowest transmittance value of 1.06. This observation is similar to the study of B. Concepción-Rosabal et al. (Concepcion-Rosabal et al. 2005). The spectra also confirm that Ag was successfully incorporated in the zeolite. The AgZ clusters in the composites may also act as scattering points that may reduce the intensity of transmitted light. This reduction provides insights on the dispersibility of the AgZ particles in the polymer matrix.

Assessment of the thermal properties of AgZ and AgZ-Ch composite samples

The TG and DTG curves of the synthesized AgZ samples after heating up to 700°C are shown in Fig. 4. Weight loss was observed to be continuous, with about 10 wt% of the AgZ sample was lost after the thermal treatment done in the range of 25 to 700°C. The onsets of degradation of AgZ, however, were determined through the endothermic peaks of the DTG curve at temperatures 45.45°C, 234.13°C and at 442.67°C. The smooth and continuous TG curve of the AgZ sample is similar to the works of Mansouri et al. (Mansouri et al. 2013) and Akdeniz and Ulku (Akdeniz & Ulku, 2008). This suggests that the structure of zeolite did not change upon dehydration. The degradation of the as-received NaZ sample, on the other hand, was observed at the range of 104°C to 662°C (Olegario-Sanchez, 2016). This indicates that the incorporation of Ag into the zeolite caused the onset of degradation to shift to lower temperatures.
Figure 5 shows the TG and DTG curves of the AgZ-Ch composites. Pure chitosan has two degradation steps. The first weight loss is observed at 60°C where this could be due to the water loss in the chitosan structure. The second major weight loss of chitosan is located at 300°C (Uygun et al. 2011). The onsets of degradation of the composites are clearly shown in the DTG curves. For the AgZ-Ch composites, three degradation steps were observed. The peak temperatures and amount of weight loss of each AgZ-Ch composites were summarized in Table 1. The addition of AgZ into the chitosan structure has shifted its onsets of degradation to lower values. The water loss is higher in 0.5% AgZ-Ch composite. Overall, the 1.0% AgZ-Ch composite has the least amount of residue remained after the analysis.
Fig. 4 TG and DTG curves for AgZ samples
Fig. 5 (a) TG and (b) DTG curves of 0.5%, 1.0%, 1.5% and 2.0% AgZ-Ch composites

<table>
<thead>
<tr>
<th>AgZ Content (%)</th>
<th>1st Step</th>
<th>2nd Step</th>
<th>3rd Step</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Temp. (°C)</td>
<td>Weight Loss (%)</td>
<td>Peak Temp. (°C)</td>
</tr>
<tr>
<td>0.5</td>
<td>60.90</td>
<td>17.49</td>
<td>167.61</td>
</tr>
<tr>
<td>1.0</td>
<td>48.55</td>
<td>13.15</td>
<td>153.71</td>
</tr>
<tr>
<td>1.5</td>
<td>46.10</td>
<td>13.49</td>
<td>171.71</td>
</tr>
<tr>
<td>2.0</td>
<td>44.37</td>
<td>12.66</td>
<td>153.33</td>
</tr>
</tbody>
</table>

Table 1: Summary of the weight loss of each AgZ-Ch composite
Fig. 6 OES spectra of 0% AgZ-Ch composites exposed at 16 Pa and 30 Pa Ar plasma discharges.
Fig. 7 OES spectra of 1.0% AgZ-Ch composites exposed at 16 Pa and 30 Pa Ar plasma discharges

Optical emission spectral analyses of the discharge

The OES analysis was used to probe the gas discharges in this work. Figures 6 and 7 show the OES of 0% AgZ-Ch and 1.0% AgZ-Ch composites exposed at 16 Pa and 30 Pa Ar plasma discharges, respectively. The intensity of the plasma discharges of the composites varied from 0 to 6500 cps which was measured at a wavelength range of 0 to 1000 nm. It can be observed that for both OES spectra of the 0% AgZ-Ch composite (16 Pa and 30 Pa), the dominating species are C, CO, N, N\textsubscript{2}, NH, O\textsubscript{2} and Ar (Navratil et al. 2006). The presence of these species may be attributed to the possible dissociation of some C, H, O and N species from the chemical structure of chitosan.
after plasma treatment. In addition, the spectra of 1.0% AgZ-Ch composites (both treated with 16 Pa and 30 Pa) revealed that Al peaks dominated the C I, and O$_2$ R I peaks at wavelengths 736 nm and 762 nm, respectively (Navratil et al. 2006). The Ag and Si peaks were also observed at 705 nm and 852 nm, respectively. In summary, additional peaks were observed to both 1.0% AgZ-Ch spectra due to the Al, Ag, and Si species (Navratil et al. 2006). This confirmed the presence of AgZ in the 1.0% AgZ-Ch composites and specifically, the presence of Ag in the zeolite minerals.

Bacteria count in the pristine and plasma-treated pure chitosan film

Bacterial count was used to determine possible presence of bacterial colonies on 0% AgZ-Ch composites after plasma treatment. The films were observed for 48 h and their washings (diluted in two concentrations- $10^{-1}$ and $10^{-2}$) were tested by counting possible presence of colonies using a Quebec colony counter. Results, summarized in Table 2, revealed that the pristine 0% AgZ-Ch composite does not have any bacterial inhabitants on its surface since less than 10 CFU/cm$^2$ was observed on the sample. This could indicate that the apparatus and glassware used for the composite fabrication were sterilized enough to avoid bacterial colonization on the surface. In addition, the soaking of the 0% AgZ-Ch composites in an isopropyl alcohol solution might have influenced disinfection on the surface of the composite. Plasma-treated 0% AgZ-Ch composites were also investigated. Plasma treatment is also considered as a
sterilization method since it relies on specific active agents, such as ultraviolet (UV) photons and radicals (Moisan et al. 2002). No more than 10 CFU/cm$^2$ was also observed in the plasma-treated AgZ-Ch composites. This revealed that plasma modification does not initiate bacterial colonization on the AgZ-Ch composites. Further investigation may be used to evaluate the effectiveness of plasma treatment for sterilization against test microorganisms.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colony-forming units (CFU/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>&lt;10</td>
</tr>
<tr>
<td>16 Pa</td>
<td>&lt;10</td>
</tr>
<tr>
<td>30 Pa</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

**Discussion**

The XRD pattern revealed that the Philippine natural zeolite used in this study was mostly composed of clinoptilolite and mordenite, followed by feldspar and quartz. The analysis confirmed that the zeolite sample used in the ion-exchange process is Na-zeolite, which is the mostly used zeolite in studies prior to ion exchange due to the high exchangeability of Na$^+$ ions when immersed in aqueous solution (Ferreira et al. 2012) (Demirci et al. 2014). The characteristic peak of chitosan did not also seem to change with the presence of AgZ particles. The absence of Ag and AgZ peaks in the XRD patterns of the composite may be due to their very small particle sizes which cannot be detected by XRD. The effect of particle size distributions for natural zeolites
was observed by Concepcion-Rosabal et al where they have determined the minimum radius limit of the characterization techniques XRD, SAXS, and UV-Vis (Concepcion-Rosabal et al. 2005). With this, XRD may be combined with SAXS and UV-Vis to allow characterizing different sizes of Ag particles (Concepcion-Rosabal et al. 2005).

In this study, UV-Vis was used, in combination with XRD, to further characterize Ag as well as AgZ particles in the composites. In our previous work, the Ag peak, expected at 37.16°, may not be clearly detected in the XRD pattern of the AgZ sample since the peak also corresponded to a zeolitic peak (Taaca & Vasquez Jr., 2017). Yet, there was an antibacterial activity observed in the AgZ sample against *E. coli* and *S. aureus* (Taaca & Vasquez Jr., 2018). According to the extensive study conducted by B. Concepcion-Rosabal et al., natural clinoptilolites with Ag (Ag-NC), reduced at 400°C, may have XRD characteristic peaks of metallic Ag between 37° to 45° (Concepcion-Rosabal et al. 2005). These peaks may be attributed to large Ag particles located outside of the clinoptilolite and mordenite channels after reduction at 400°C. Furthermore, a study conducted by Bogdanchikova et al. comprehensively showed the several types of Ag clusters in zeolites with different molar SiO₂/Al₂O₃ ratios (Bogdanchikova et al. 1999). Different Ag clusters are assigned to different optical spectral wavelength peaks. The peak at 290 nm is designated to the clusters of positively-charged Ag₈ᵐ⁺ or Ag₇ᵐ⁺ (Bogdanchikova et al. 1999). In this study, the transmittance peak at 290 nm was observed to decrease with increasing AgZ content. The disappearance of the transmittance peak can denote that the absorbance increases as AgZ content increases. This then indicates that the formation of Ag₈⁺, due to the
reduction of the Ag$_8^{m+}$ and Ag$_7^{m+}$, becomes more defined and stabilized at higher contents of AgZ. Further, this study observed that calcination as a reduction process to Ag particles allowed the formation of several Ag clusters inside the zeolite pores and can only be observed by UV-Vis spectroscopy.

Aside from UV-Vis, OES was also utilized where an Ag I peak was observed at 705 nm. The detection of Ag peak after plasma treatment could signify that the type of Ag present in the AgZ-Ch composites is from the cluster of positively-charged Ag$_8^{m+}$ or Ag$_7^{m+}$. The plasma treatment done in this study utilized low pressures yet OES analyses displayed an Ag peak at low intensities than 1500 cps, implying that Ag ions were not completely reduced, by calcination, to Ag$_0^0$ since some remained in their positively-charged states. With the analyses from UV-Vis and OES, it is recommended that the calcination step must be improve or other reduction process may be utilized such as plasma treatment (Nolan et al., 2018) (Osonio & Vasquez, 2018) to reduce Ag$^+$ ions to Ag$_0^0$ particles. The Ag$_0^0$ state is more desired for antibacterial applications as it has morphological and physicochemical characteristics which influence its antibacterial activity aside from its ability to control the release of ions (Dizaj et al. 2014).

Thermal analysis of the AgZ samples revealed that the degradation of samples was caused by dehydroxylation and dehydration, similar to Mansouri et al. 2013. The observed onsets of degradation can be attributed to weight losses from hygroscopic water, loosely bonded water, and hydroxyl (-OH) groups existing in the structure of clinoptilolite, respectively (Mansouri et al. 2013). The onsets of degradations of AgZ,
moreover, indicate that these minerals are suitable for biomedical applications. The results show that the AgZ synthesized from the ion exchange method did not reveal any changes in the phase composition and chemical structure of zeolites. The effect of Ag was observed in the TGA result where the thermal degradation of AgZ is at lower temperature compared to NaZ. Despite this decrease, compared to the pure chitosan sample, the composites still meet the thermal requirement for a biomaterial or an implant. Since the first onsets of degradation are found at the range of 44.37-60.90°C, the composites are proven to have a good thermal stability and can support physiological functions inside the human body where the normal temperature is around 37°C.

Sterilization is a method of eliminating, either physically or chemically, all types of microorganisms (Moisan et al. 2002). One of the sterilization techniques used is through plasma sterilization which relies on photons and radicals to actively react with different microorganisms. Plasma treatment was done on the AgZ-Ch composite to modify surface properties such as wettability and surface roughness, which was discussed in our previous work (Taaca & Vasquez Jr., 2017). In this study, possible effect of plasma treatment as a sterilization technique was observed. The test revealed that no more than 10 CFU/cm² of colonies was observed at all samples. This denotes that the AgZ-Ch composite can readily be used as an implant without worrying its sterility since bacterial colony is at minimum. Same can be deduced with plasma-treated AgZ-Ch composites since all samples were soaked in an isopropyl alcohol solution prior to plasma treatment. However, it is recommended that the effect of
plasma treatment as a sterilization technique must be further investigated for this particular material.

CONCLUSIONS

In this study, AgZ-Ch composites were fabricated using a solvent-casting approach. The zeolite sample used in this study was confirmed to be mostly composed of Na-clinoptilolite with traces of mordenite, quartz and feldspar, as seen on the XRD pattern. With the presence of Na-clinoptilolite, the ion-exchange process was more successful since Na\(^+\) ions are known to be easily exchanged when immersed in an aqueous solution. UV-Vis analysis exhibited a decrease in the transmittance peaks especially at 290 nm, implying that AgZ particles are present in the AgZ-Ch composites. Moreover, the decrease in the transmittance peaks denotes that there is an increase in the absorbance at 290 nm and thus entailing that the reduction of Ag ions becomes more defined and stabilized for higher concentrations of AgZ. OES spectra displayed an Ag I peak at 705 nm in lower intensities suggesting that some Ag present in the composites were not reduced and therefore, being dissociated from the structure of the composite when exposed to the Ar plasma discharge. Thermal and sterility properties of the AgZ-Ch composites were also observed. The presence of AgZ in the composite caused the onsets of degradation to occur at lower temperatures but higher than 37°C, which is the typical temperature of a human body. Results of the bacterial count reveal that the AgZ-Ch composites, both pristine and plasma-treated, are safe to
use as a biomaterial applied either topically or as an implant since the surface of these composites do not serve as habitats of microorganisms. With these results, the synthesized AgZ-Ch composites, both pristine and plasma-treated, are observed to meet the minimum requirements as a biomaterial.

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