

Coal Fly Ash-based Geopolymer Spheres Coated with Amoxicillin and Nanosilver for Potential Antibacterial Applications

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Geopolymers are emerging “green” materials for its low embodied energy and carbon footprint, and its potential to valorize wastes, such as coal fly ash. It is an inorganic cementitious material formed from the polymerization of aluminosilicates in an activating solution such as that of alkali hydroxides or silicates. Their superior mechanical properties, including heat and fire resistance make them an excellent material for diverse applications. Recent studies have also exploited the tunable open porosity and adsorbing properties of geopolymers. Our work thus explores the potential of porous geopolymer spheres for antibacterial applications. These spheres were synthesized using coal fly ash as the geopolymer precursor and the porous surface is coated with either amoxicillin or silver nanoparticles (AgNPs) adsorbed in the matrix. For the AgNP geopolymer spheres, SEM images show spherical nanostructures when using ascorbic acid as a reducing agent, while spherical, cubical, and wire structures were observed when reduced using NaBH₄. Indication from UV-Vis results also suggests the gradual release of both amoxicillin and AgNPs in the solution from the functionalized geopolymer spheres. Using *E. Coli* as the test organism for a modified disk diffusion assay, both showed zones of inhibition against the bacteria. Further tests on

antibacterial application of AgNP geopolymer spheres show their effectiveness to kill at least 95% of the *E. coli* in a water sample initially containing 10^5 cfu/mL in just 30 minutes.

Keywords: Antibacterial, Geopolymer, Coal Fly Ash, Amoxicillin, Silver Nanoparticles

INTRODUCTION

Geopolymer has been recently recognized as an emerging alternative cementitious material to replace Portland cement for its low embodied energy and carbon footprint, and its potential to valorize waste such as coal fly ash, among others. Davidovits originally coined the term “geopolymer” in the 1970s to refer a class of material whose molecular structure consists of an amorphous to semi-crystalline inorganic network of mineral molecules (Davidovits 1991). They are formed from the polycondensation of aluminosilicates found for example in coal fly ash while reacting in an activating solution such as that of alkali hydroxides or silicates. Mechanical properties such as compressive and flexural strengths are at par with traditional cements, but have exceptional fire and chemical resistance. With these specific properties, numerous researches today focus on the use of geopolymers as a more environmentally friendly construction material (Singh et al. 2015). Moreover, recent studies on geopolymers have started to investigate its applications for other industries, particularly in refractory materials (Temuujin et al. 2011), in soil stabilization (Tigue et al. 2018), and lining for cement pipes to increase their durability (Chindaprasirt and Rattanasak 2016),

among others.

Porous geopolymers are also being developed which are characterized by their large surface area and tunable pore sizes that can be utilized as cost-effective replacements for different media since they can be used as adsorbents, catalysts, sensors, filters, and thermal insulators (Asl et al. 2018). In addition, a study by Armayani et al. in 2017 determined the antibacterial property of a silver nanoparticle modified geopolymer composite to inhibit growth of certain types of bacteria that is applied as an antibacterial coating material for walls and floors. Another related study by De Muynck et al. in 2008 utilized geopolymer mortars by making them antimicrobial by adding triclosan and benzalkonium chloride. Results show that the geopolymer mortar with triclosan inhibits the growth of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus*, where large zones of inhibition were observed, indicating that antimicrobial concrete mixtures are feasible and can be used to improve the hygienic conditions of various environments.

In this study, porous geopolymer spheres based from coal fly ash and waste pen shells (De La Cerna et al. 2018) were enhanced with antibacterial properties by coating the porous surface with amoxicillin

and silver nanoparticles (AgNPs). Morphological characterizations were also done using scanning electron microscopy (SEM). In addition, the dissociation of these antimicrobial geopolymers in solution were measured using UV-Vis spectrophotometry. Antibacterial activity test of these geopolymer spheres were also done using *E. coli* as the indicator.

MATERIALS AND METHODS

Preparation of Geopolymer Spheres

Geopolymer spheres were synthesized using a modified method described in Tang et al. 2015. This study used coal fly ash and calcined waste pen shells as the main raw materials. Foaming agents were added as well to increase the porosity of the spheres. The fly ash and the calcined shells were first combined at a total weight of 80 g to act as a geopolymer precursor and form the spherical beads. An amount of 7.5 g of a 12 M NaOH solution was added with 30.0 g of WGS to form the alkali activator solution. The solid precursor was then added to the alkali activator and stirred by hand for 1 minute. 1.7 mL of water was also added, then hand-stirring was done for another 2 minutes. Finally, varying masses of a 50% H₂O₂ solution was dropped in, ranging from 0.15% to 0.35% of the mass of the geopolymer. This was stirred thoroughly for another 2 minutes. The resulting paste was brown and had a thick consistency.

The geopolymer paste was then loaded into a syringe and injected dropwise into a hot PEG-600 solution at 90°C. The dropwise addition of the paste,

coupled with the high temperature of the medium, allows the paste to rapidly solidify into 4-5 mm beads. The formed spheres were left in the PEG-600 for about 20 minutes in order to fully harden; they were then collected, rinsed thoroughly, and left to cure in a laboratory oven at 90°C for another 24 hours. Fig. 1 shows the synthesized geopolymer spheres



Fig. 1: Geopolymer spheres from coal fly ash and calcined waste pen shells

Coating of Amoxicillin in Geopolymer Spheres

In a 15 mL centrifuge tube, a solution of 1 gram of amoxicillin dissolved in 5 mL of distilled water was prepared. The geopolymer spheres were immersed in this solution for 24 hours. They were then removed from the solution and were allowed to air dry at room temperature for another 24 hours.

Coating of Geopolymer Spheres with AgNPs

This method of coating geopolymer spheres with AgNPs was based on the study by Banne et al. 2017 and was modified to use geopolymer spheres as adsorbent media. Three sets of

geopolymer spheres were rinsed thoroughly with distilled water and were left to dry in open air at room temperature. Next, the geopolymer spheres were submerged in 1 mL of 1 M AgNO_3 inside a microcentrifuge tube for 1 hour. Afterwards, the AgNO_3 solution was discarded from the spheres, and another 1 mL of 1 M AgNO_3 was added and left to submerge for another hour. Then, the geopolymer spheres were transferred in a petri dish to air dry.

To produce AgNPs, *in situ* reduction of AgNO_3 inside the treated geopolymer spheres was done by submerging one set in 1 mL of 1 M NaBH_4 , and another set in 1 mL of 1 M ascorbic acid, both for 1 hour. To confirm the presence of AgNPs in the reducing agent treated geopolymer spheres, surface morphology was determined using scanning electron microscopy and was compared to the third set in which no reducing agent was added which corresponds to the control sample.

Dissociation Efficacy of the Geopolymer Spheres via UV-Vis Spectrophotometry

A. Amoxicillin Coated Geopolymer Spheres

The ability of the loaded spheres to function as antibacterial agents highly depends on its dissociation activity. Thus, it is essential to quantitatively determine the amount of amoxicillin dissociated from the loaded spheres. This was done by determining the lambda max of the antibiotic solution using a Hitachi U-2900 UV-Vis spectrophotometer in which dilutions of the mixture were scanned 800-200 nm. With this, the absorbance values

of the amoxicillin dilutions were used for the construction of the calibration curve.

In a Falcon tube, the amoxicillin loaded geopolymer spheres were immersed in 5 mL of distilled water together with a magnetic stirrer. The contents of the tube were then mixed for three minutes. Then, a 3 mL portion of this mixture was transferred to a quartz cuvette to measure its absorbance at the 500-200 nm range, with distilled water as the reference. After the analysis, the contents of the cuvette were put back in the Falcon tube and continued mixing for another 3 minutes before measuring the absorbance again. This procedure was repeated until a total of 30 minutes of mixing time was achieved.

B. AgNP Functionalized Geopolymer Spheres

The same procedure was done to the geopolymer spheres with adsorbed AgNPs, except a single geopolymer sphere was immersed in 4 mL of distilled water and the contents of the tube were shaken mildly at a constant motion. Every 3 minutes for 30 minutes, the absorbance of the solution was recorded, with wavelengths ranging from 600 nm to 250 nm.

Antimicrobial Test of the Geopolymer Spheres

A. Modified Standard Disk Diffusion Technique

Their bactericidal effect against *E. coli* using a modified standard disk diffusion technique was used in the following geopolymer sphere samples:

NaBH₄ reduced and ascorbic acid reduced AgNPs, with AgNO₃ only, and the amoxicillin loaded samples.

Prior to antibacterial testing, Mueller-Hinton agar (MHA) was prepared according to the standard protocol of Clinical & Laboratory Standards Institute (CLSI) in which 38 g of agar were suspended per 1000 mL of distilled water. The mixture of agar and distilled water was heated to ensure homogeneity before being autoclaved. After sterilization, the agar mixture was cooled to 45-50°C, mixed well, and was poured into sterile petri dishes.

The bacteria, *E. coli*, were first transferred into a cooled flask containing the MHA before being poured into respective petri dishes. Afterwards, instead of using standard paper disks. For each plate, four geopolymer spheres were submerged in the MHA before it completely solidifies. The plates contain approximately 1.5×10^8 suspension of *E. coli*. The amount of *E. coli* was determined using 0.5 McFarland Standard. The zones of inhibition the geopolymer spheres showed after 24 hours of incubation in 37°C were then recorded using a Vernier caliper.

B. Antimicrobial efficiency of AgNP-geopolymer spheres in E-coli contaminated water

A 10^5 cfu/mL inoculum was first prepared using a culture of *E. coli* made 24 hours prior to the experiment. The 30 mL of this inoculum was placed in a beaker containing NaBH₄ reduced AgNP-geopolymer spheres. The beaker was then

agitated in an orbital shaker set at 300 rpm. Exactly 1.5 mL of 5% w/w solution of sodium thiosulfate was added 30 minutes after. This immediately halts the antimicrobial activity of the silver ions, ensuring a constant contact time of 30 minutes was done for all of the samples. Then, 1 mL of the liquid from each sample and their serial dilutions (up to 10^{-3}) were plated in agar and left to incubate at 37°C for 24 hours. Plate counting was then done to determine the final concentration of *E. coli* in the water. Note that this plate counting technique was also done to determine the initial *E. coli* count of the water.

RESULTS AND DISCUSSION

Coating of Geopolymer Spheres with AgNPs

Geopolymer spheres coated with AgNPs were synthesized in situ by the reduction of AgNO₃ with either NaBH₄ or ascorbic acid. Investigation of the geopolymer spheres through SEM shows distinct differences in their morphologies. The untreated geopolymer spheres, as shown in Fig. 2, are immersed only in distilled water. This control sample shows smooth areas that make up the surface of the neat geopolymer.

The geopolymer spheres with AgNPs from the reduction by ascorbic acid produced spherical silver nanostructures (Fig. 3a) with uneven densities of the AgNPs surrounding the geopolymer, while the ones reduced by NaBH₄ also produced spherical shapes, alongside with uneven cubic nanostructures, and silver nanowires (Fig. 3b).

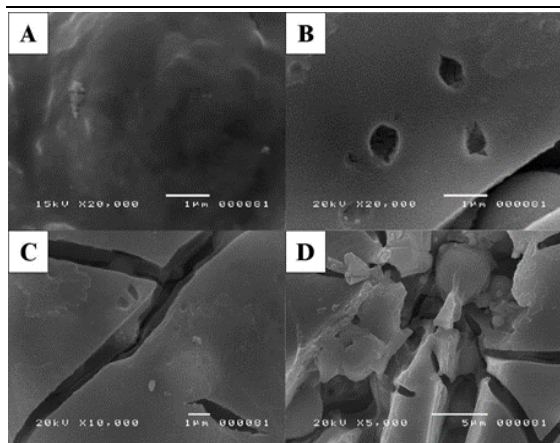
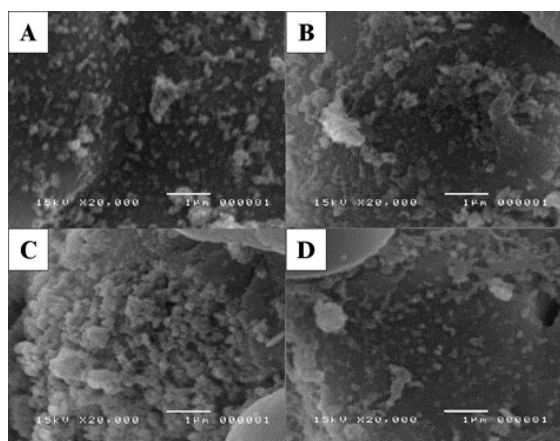
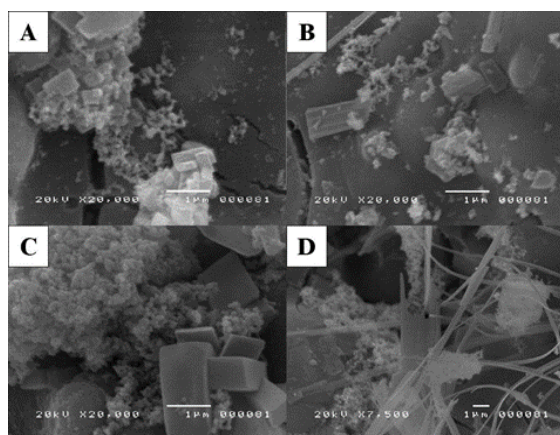


Fig. 2: SEM Micrograph of geopolymer spheres immersed only in distilled water.



(a)



(b)

Fig. 3: SEM micrographs of geopolymer spheres with AgNPs prepared by reducing 1M AgNO_3 (a) with 1M ascorbic acid (b) with 1 M NaBH_4 .

The spherical nature and the varying densities and sizes of the AgNPs formed by reduction of ascorbic acid was suspected to be due to the absence of a stabilizing agent during the reduction process. This was supported from a study by Al-Thabaiti et al. in 2013, in which the role of stabilizers in the formation of nanostructures are important. It was found out that the presence of stabilizers or surfactants, such as cetyltrimethylammonium bromide (CTAB), leads to an increased formation of monodisperse spherical nanostructures.

On the other hand, NaBH_4 , generated AgNPs with varying morphologies on the surface of the geopolymer spheres. Since NaBH_4 is the stronger reducing agent, its reaction to form AgNPs are rapid as compared to reduction by ascorbic acid. This rapid reduction does not allow proper nucleation of the AgNPs and instead forms various sizes and morphologies (Khodashenas & Ghorbani 2015).

Another possibility to consider is that the geopolymers contain other reagents used for its synthesis. Polyethylene glycol is used to synthesize geopolymer spheres. A study by Cong et al. noted that silver nanowires may be synthesized by reducing AgNO_3 in polyethylene glycol (PEG) with poly (vinyl pyrrolidone) (PVP) as a capping agent (Cong et al. 2012). Also, a similar study was introduced by Sun and Xia in 2002, wherein they synthesized cubic AgNPs by reducing AgNO_3 with ethylene glycol in the presence of PVP. It is possible that remnants of the PEG-600 solution may have remained in the geopolymer due to

the sphere's porosity, even though they were thoroughly washed, thus affecting the development of the AgNPs, and eventually creating silver nanowires and cubic AgNPs in the process.

Dissociation Efficacy of the Geopolymer Spheres via UV-Vis Spectrophotometry

For the geopolymer spheres to become effective antibacterial carriers, it is important that the functional materials would adhere to its structure longer. Since its porosity allows components to incorporate themselves within the geopolymer, it may also readily allow the antibacterial agent to leach or dissociate from it.

A. Amoxicillin Coated Geopolymer Spheres

The lambda max of the antibiotic mixture was determined to be at 350 nm. Thus, absorbance values of the amoxicillin dilutions at 350 nm were obtained to get the calibration curve as shown in Eq. 1 and Fig. 4.

$$y = 0.0752x - 0.0042 \quad (1)$$

Using the calibration curve, the concentrations of the diffused amoxicillin per time was obtained. Since the concentration is directly proportional to the absorbance values, this means that as the geopolymer spheres spend more time mixing in the solution, the more antibiotic is dissociated over time as denoted by its increasing trend (Fig. 5). Furthermore, the dispersion of the drug is gradual; the antibiotic did not completely dissociate all

at once, as the spheres were immersed in water. The chemical structure of the amoxicillin may have contributed to this phenomenon as it contains different functional groups which are capable of hydrogen bonding to the geopolymer network, hindering the full release of the drug. The steady release of the drug over time the idea that the geopolymer sphere is a possible drug carrier.

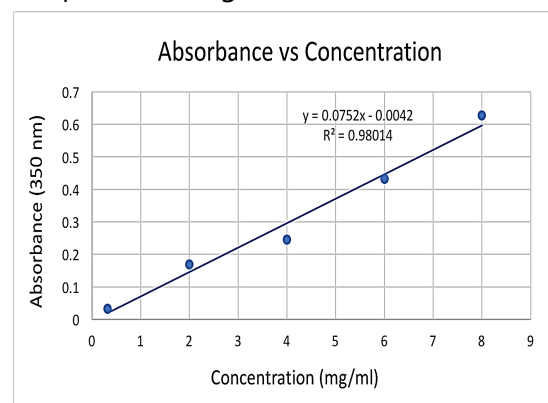


Fig. 4: Absorbance versus concentration calibration curve of the diluted amoxicillin solutions read at 350 nm.

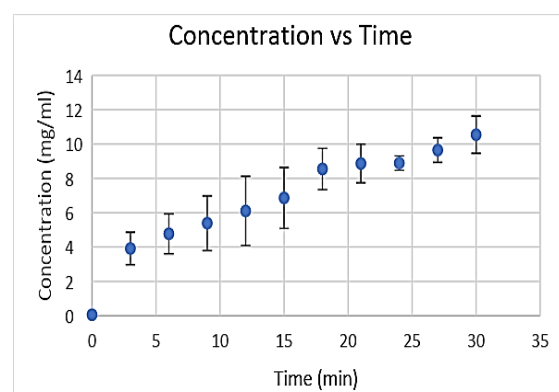


Fig. 5: Graph of average concentration values of dissociated amoxicillin over the course of 30 minutes.

B. AgNP Coated Geopolymer Spheres

To test the geopolymer spheres' efficacy to contain the AgNPs, the absorbance of distilled water immersed

with AgNP geopolymer spheres reduced with NaBH_4 over 30 minutes was measured.

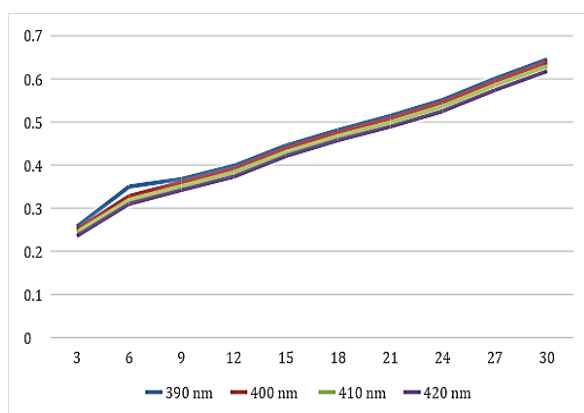


Fig. 6: Absorbance of distilled water with AgNP geopolymer reduced by NaBH_4 from 390-420 nm for 30 minutes

The solution was scanned in the 600-250 nm range and comparison of values shows a significant increase in absorbance after 30 minutes. The absorbance peak, set at 250 nm, increased from 0.377 to 0.95 after half an hour. According to a study by Tolve et al. in 2015, AgNPs have a peak absorbance in the wavelength range of 390 to 420 nm.

It is seen in Fig. 6 that the absorbance of the distilled water solution exposed to the functionalized geopolymer spheres has a linear relationship with as time increases. Furthermore, the dispersion of the AgNPs is gradual as the length of mixing time increases; the AgNPs did not completely dissociate all at once, as the spheres were immersed in water.

Preliminary Antimicrobial Testing

A. Geopolymers Spheres with AgNPs

To determine the antibacterial

efficacy of the geopolymer spheres impregnated with AgNPs, the zone of inhibition (ZOI) against *E. coli* was determined using an antibacterial susceptibility test. AgNP geopolymer spheres reduced using NaBH_4 displayed significant ZOIs from 7-10 mm, showing that these geopolymers were successful in inhibiting the growth of bacteria (Fig. 6a). On the other hand, the synthesized AgNP geopolymer spheres reduced using ascorbic acid did not show any ZOIs (Fig. 6b).

A known cause of AgNPs' antibacterial activity is its ability to increase the bacterial cell's permeability. Due to this, the cell fails to regulate the transport of substances between its membrane, eventually leading to its death. These nanoparticles accumulate in the cell membrane and form pits in the cell wall (Stoimenov et al. 2002, Sondi & Salopek-Sondi 2004, Li et al. 2010). Also, the silver particles disrupt the replication of bacterial DNA by the diffusion of Ag through the cell membrane which allows it to react with and inactivate thiol containing proteins. This then allows Ag to bind to DNA, thus inhibiting replication (Feng et al. 2000). Further studies show that treatments of AgNPs induced a direct effect over the cell's mitochondrial function associated with the generation of reactive oxygen species, caspase-3 activation and DNA fragmentation based on their size (Sriram 2012).

The size of the AgNPs also greatly affect its cytotoxicity. Smaller particles are shown to have more efficient antibacterial activity as compared to larger particles (Baker et al. 2005). The synthesis of AgNPs

by reduction with NaBH₄ generally yields smaller particles (Pyatenko et al. 2007) which is mainly due to the reducing power of NaBH₄. The reduction by the weaker reducing agent, ascorbic acid, yielded clumped and agglomerated structures (Fig. 2a) which ultimately affected its antibacterial activity.



(a)



(b)

Fig. 7: Zones of inhibition of *E. coli* exposed to AgNP adsorbed geopolymer reduced by (a) NaBH₄ (b) ascorbic acid

It was also found out that while reduction by ascorbic acid indeed synthesized AgNPs, it is inefficient in keeping them inside the geopolymers as shown in Fig. 6b. It showed no ZOIs, but instead a discoloration of the agar medium due to the leached Ag. Another trial, wherein the geopolymers were only soaked in AgNO₃ and then tested against *E. coli*, formed uneven ZOIs (Fig. 7) and a brown discoloration was also detected, primarily due to AgNO₃.

One-way analysis of variance was then used to determine if there is a statistically significant difference between the ZOIs yielded by AgNP geopolymer reduced using NaBH₄, ascorbic acid, and control geopolymers spheres. Since the *p*-value (1.23 e-19) is lower than the significance level of 0.05, the null hypothesis is rejected. Thus, there is a significant difference between the ZOIs of the NaBH₄ reduced AgNP treated geopolymer compared to those untreated samples.

In addition, the NaBH₄ reduced AgNP-geopolymer spheres were evaluated for antimicrobial efficiency in a *E. coli* contaminated water using Eq. 2.

$$AE = \frac{\text{initial count} - \text{final count}}{\text{initial count}} \times 100\% \quad (2)$$

Table 1 summarizes the results from this preliminary anti-microbial test after the spheres are in contact with the *E. coli* contaminated water for 30 minutes. It is evident from the results that majority of the trials were successful in eliminating at least 95% of the *E. coli* in the water sample.

Table 1. Antimicrobial Efficiencies (AE) of AgNP-geopolymer spheres

Trial	AE (%)
1	95.46
2	97.97
3	98.88
4	98.65
5	99.94

B. Geopolymer Spheres with Amoxicillin

Antibacterial testing is also a means to determine the drug carrying efficiency of the geopolymer spheres. For the spheres to be a candidate as a potential carrier, no adverse reaction between the drug and the geopolymer should occur.

The geopolymers were autoclaved after immersion in antibiotic solution and prior to exposure to *E. coli* in Mueller-Hinton agar; 1.0 g/5 mL was the dosage used for the antibiotic solution to assure immediate results. After 24 hours of incubation, no ZOI were observed and bacterial growth were present around the spheres, and is possibly due to the evaporation of the antibiotic during the process of sterilization through autoclaving.

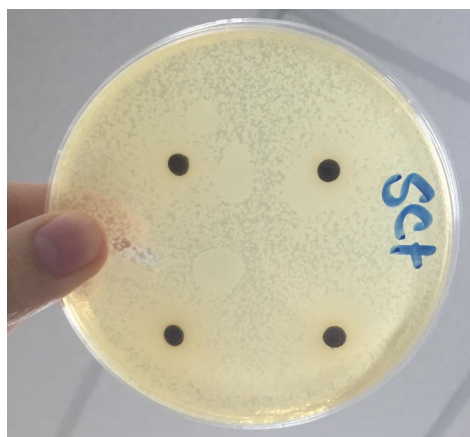


Fig. 8: Zone of inhibition of the amoxicillin loaded and autoclaved geopolymer spheres in *E. coli*.

Autoclaving resulted in the degradation of amoxicillin from the geopolymer spheres. Thus, by omitting this step, the antibiotic should remain to be loaded into the geopolymer. All the

geopolymer spheres were able to exhibit significant ZOIs at around 19-20 mm (Fig. 9).

Through a one-way analysis of variance, it was observed that the p-value (3.217×10^{-11}) is lower than the significance level of 0.05. Thus, the null hypothesis is rejected, meaning that there is a significant difference between the treated and the untreated spheres.

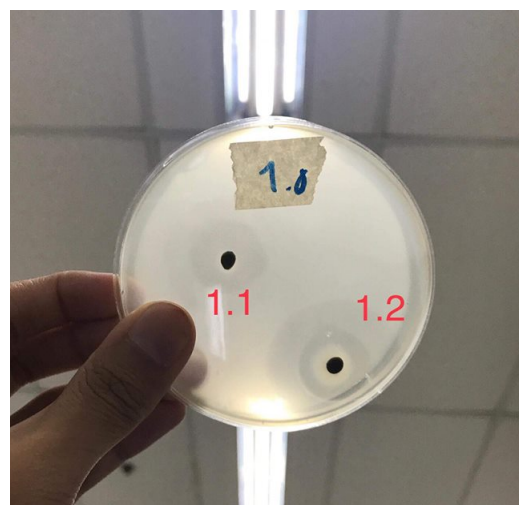


Fig. 9: Zone of inhibition of the amoxicillin loaded and unautoclaved geopolymer spheres in *E. coli*.

After incubation, bacterial growth was observed near the ZOIs (Zone of Inhibitions). This can be accounted to the amoxicillin being completely dissociated. If amoxicillin were not completely dissociated, no bacterial growth should have occurred. With this it can be inferred that the geopolymer spheres possess the ability to effectively carry and fully deliver the drug loaded unto it, allowing them to be promising drug carriers.

CONCLUSIONS

Coal fly ash based geopolymer

spheres were coated or loaded in their porous surface with AgNPs and amoxicillin to exhibit antibacterial properties which are determined via susceptibility tests against *E. Coli*. This study determined that the geopolymer spheres with amoxicillin displayed a larger ZOI as compared to the spheres with AgNPs reduced by NaBH₄. Meanwhile, AgNPs reduced by ascorbic acid showed no ZOIs due to its weak reduction strength and have agglomerated AgNPs as shown in SEM. Spheres with AgNPs reduced by NaBH₄ were tested for antimicrobial efficiency and shows to kill at least 95% of the *E. coli* in a water sample initially containing 10⁵ cfu/mL in just 30 minutes. Note that these only aim to serve as preliminary studies and future work will be done to further investigate the potency of their antibacterial effect. Nevertheless, both applications show gradual dissociation of the added functionalities in the geopolymer spheres as determined by UV-Vis spectroscopy. This shows also that the coal fly ash based geopolymer spheres can be used as a carrier medium for AgNPs and amoxicillin for potential antimicrobial applications.

ACKNOWLEDGMENT

The authors would like to acknowledge the financial support from the Department of Science and Technology (Philippines) through their Engineering Research and Development for Technology (ERDT) scholarship program and Philippine Council for Industry, Energy, and Emerging

Technology Research and Development – Advanced Device and Materials Testing Laboratory (PCIEERD-ADMATEL) grant, and URCO Research Project.

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