UNIVERSITY of SAN CARLOS
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CHE 4119L FORM-1:
Undergraduate Research Proposal Template
Endorsement Sheet

Department of
Chemical Engineering
Talamban, Cebu City, Philippines 6000

Term/Academic Year:
First Semester AY 2021-2022

Research Proposal Endorsement and Approval

I/we have read and agreed to the content of the research proposal entitled

CHITOSAN-SILVER NANOCOMPOSITE AS ANTIBACTERIAL COATING FOR MOBILE PHONE GLASS PROTECTORS

Prepared and submitted by

CANAMA, GIBSON JAKE C.

DELCO, MONICA CLAIRE L.

TALANDRON, RHOEL A.

I/we affirm that the same complies with the standards prescribed for the research proposal requirement.

In view thereof, I/we hereby endorses the said research proposal for review and oral presentation.

Endorsed By:

NOEL PETER B. TAN, Ph D.
Name and Signature of Adviser

Date Endorsed

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CHITOSAN-SILVER NANOCOMPOSITE AS ANTIBACTERIAL COATING FOR MOBILE PHONE GLASS PROTECTORS

REVIEW

Agapay, Ramelito C. Name and Signature of Reviewer	Siacor, Francis Dave C. Name and Signature of Reviewer
Tampus, May V. Name and Signature of Reviewer	Napisa, Victoria F. Name and Signature of Reviewer

November 23, 2021
Date of Review

APPROVAL

Agapay, Ramelito C. Name and Signature of Primary Reviewer	Date Approved
Siacor, Francis Dave C. Name and Signature of Primary Reviewer	Date Approved
Tampus, May V. Name and Signature of Primary Reviewer	Date Approved

Department of
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Title	Chitosan-silver nanocomposite as antibacterial coating for mobile phone glass protectors
Name(s) of Proponents	[1] Canama, Gibson Jake C. [2] Delco, Monica Claire L. [3] Talandron, Rhoel A.
Objectives	This research project aims to study a green synthetic route to chitosan-silver (ChAgNPs) nanocomposite with antibacterial property on mobile phone glass protectors. The objectives of this research are to: 1.) investigate the effects of chitosan concentration on the produced ChAgNP nanoparticle size upon synthesis completion. 2.) determine the optimal ChAgNP nanoparticle size (i.e., that falls within the range of 1 nm to 100 nm) by selecting the smallest acceptable size, and 3.) evaluate the antibacterial activity of the ChAgNP coating against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* at various contact times (1 minute, 1 hour, 2 hours, and 24 hours).
Project Duration	August 2021 to May 2022
Project Cost	Php 89,423.34

I. Theoretical Background

Fomite contamination is defined as the phenomenon in which bacteria and viruses transmit and remain on surfaces which may lead to infections upon human contact. Reynolds, et al. (2007) cited that the contamination of surfaces can spread infectious doses of pathogens wherein an indirect transmission to the mouths of individuals can be expected upon close contact with the infected surface. Furthermore, the risk of obtaining diseases from these microorganisms is affected by several factors such as site of contamination, excretion of pathogen to host, likelihood of pathogen to transfer to contracted individual, immunocompetence of the individual, virulence of the organism, and control measures done by the individual such as hygiene and disinfection.

1.1 Bacterial infections from fomite transmission through mobile phones

Bacterial infections are a globally concerning issue affecting the health and safety, morbidity, and mortality of everyone. These infections can progress through fomite transmission of microbe including bacteria and viruses. For several years, mobile phones have become an indispensable gadget for everyone. Thus, being a high-touch surface, mobile phones can act as a reservoir of disease-causing microorganisms and be a source for transmission once touched by hands (Pal et al., 2015).

In hospitals, for example, where people expose their phones in public, there is a high risk of contracting bacteria and viruses and, consequently, infecting those users and anyone who gets exposed to the contaminated phones. Therefore, remediating solutions to spreading infection through mobile phone glass screens is important nowadays.

I.1.1 Usage of mobile phones in hospital setting

Communication is one of the primary reasons why mobile phones are used in hospitals, minimizing delay in responding to patients' needs which is most crucial in emergency situations. Such growing interest has been observed in a review by Al Thomairy et al (2015) in which medical departments such as internal medicine, paediatrics, radiology, obstetrics, and surgery have shown a rise of usage of mobile phones and software applications to facilitate real-time

decision-making and to collect more information to grasp more knowledge of the different facets of treatment. Such device has also paved the way in improving work efficiency in hospitals by allowing rapid response to patient needs and thus providing a positive impact to patient care.

Due to the risk posed by mobile phones as a route of nosocomial or hospital-acquired infection, the contamination of these devices has become a popular object of study. In a tertiary care setting, participants consisting of healthcare workers and non-healthcare workers exhibited a mobile phone bacterial contamination rate of 81.8%. Also, 80% of their hands showed growth of bacterial pathogens. Alarmingly too, the healthcare workers particularly all had contaminated mobile phones and hands. The most predominant bacterial species identified were Coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, *Acinetobacter species*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas* species and *Enterococcus* species (Pal et al., 2015). Similar results were also reported by studies conducted in hospitals in Turkey and Nigeria (Abhinav Singh, 2012).
Distressingly, these pathogens, if not disinfected, can permeate on dry inanimate surfaces for as long as several months (Kramer et al., 2006).

I.1.2 Staphylococcus aureus

Staphylococcus aureus (S. aureus) is a gram-positive bacterium that is responsible for a wide variety of human clinical manifestations. As a gram-positive bacterium, it has a thick peptidoglycan layer and no outer lipid membrane (Steward, 2019). It occurs and infects both in community-acquired and hospital-acquired settings. It is found in the environment and in normal human flora such as in the skin and in the mucous membranes, able to cause only mild skin and soft tissue infections. However, once it enters the bloodstream and internal tissues, it can cause highly serious infections ranging from food poisoning to osteomyelitis, endocarditis, and toxic shock syndrome (Reddy et al., 2017; Taylor & Unakal, 2017).

S. aureus colonization tends to be higher in populations such as health care workers, and transmission is largely mediated by their hands. In a study by McBryde et al. (2004), contacts between health care workers and an infected patient resulted in transmission through hands or

gloves at a rate of 17%. It is mostly transmitted through direct contact (or by fomites) but could also be transmitted through other methods like sharing of contaminated objects such as needles and inhaling infected droplets from coughing or sneezing (Bush, 2018; Taylor & Unakal, 2017). *S. aureus* can live up to 7 months on dry surfaces (Kramer et al., 2006).

I.1.3 Escherichia coli

Escherichia coli (E. coli), a gram-negative bacterium, is a common cause of infections among hospitalized patients and also generally in the community (Hartl & Dykhuizen, 1984). Gram-negative bacteria have a thin peptidoglycan layer and an outer lipid membrane (Steward, 2019). Alarmingly, in the past several years, this bacterial pathogen has grown naturally resistant to penicillin and to multiple antimicrobials (Olorunmola et al., 2013; Reinthaler et al., 2003). *E. coli* causes diarrheal diseases, urinary tract infection, and neonatal bacterial meningitis in all age groups (Hartl & Dykhuizen, 1984).

E. coli can survive on dry surfaces for as long as 16 months (Kramer et al., 2006). In the hospital setting, the efficiency of this bacterium in transferring from the nitrile material of gloves to polypropylene plastic can be as high as 49% (for drug-resistant strains) and 45.9% (for drug-sensitive strains). Thus, infection through fomite transmission from an infected gloved hand to a mobile phone and eventually to the face or mouth is very possible (Takoi et al., 2019).

Although lessened, the risk of fomite transmission of bacteria in the general, non-hospital setting should not be ignored. As a common handheld device and high-touch surface, mobile phones can still be a source of infection even to the regular person. Thus, the need for antibacterial technologies for disinfection is a common one.

1.2. Current technologies on electronic device disinfection

Disinfection is one of the measures taken by industries to mitigate the spread of these microorganisms on surfaces and decrease fomite contamination. Several surfaces have been studied for disinfection with various formulations used to kill pathogens. However, with the growing advancement of technology, mobile phones are now prone to contamination especially

in clinical settings. These microbes may include antibiotic-resistant bacteria that are associated in hospital-acquired infections (Simmonds et al., 2020).

Thus, two disinfection methods are introduced and used in the industry to mitigate the spread of nosocomial bacteria in hospitals. Such methods are explained in the next two subsections.

1.2.1. Alcohols and disinfecting sprays

The most popular tools and technologies used for disinfection of electronic devices include sanitizer sprays, self-cleaning coatings, disinfectant wipes, and even UV light sanitizing devices. The use of alcohols and alcohol-based disinfectants have great potential in killing bacteria and viruses on human skin. The antibacterial mode of action of alcohols is carried out by the denaturation of proteins found in bacteria (Dastider et al., 2020). Furthermore, the use of around 70% or less (volume/volume) alcohol concentration would suffice in disinfecting electronic devices.

Even though alcohol can be used to clean and disinfect screen protectors, frequent use of alcohol onto screen protectors on a regular basis is harmful to the screen protector. Mega Mobile Incorporated (2018) reported that repeated applications of alcohol will strip the glass's oleophobic layer. Saharacase (2018), on the other hand, concluded that the anti-fingerprint coating on tempered glass will be removed when using harsh cleansers and chemicals, such as alcohol. The oleophobic layer is meant to reduce fingerprints and to give a matte-type smoothness to the screen (Brookes, 2020). Besides aesthetics, the accumulation of oil on the screen surface promotes the adhesion of microbes, including bacteria, on to the screen (Rosenberg et al., 1991).

Hence, the common method of alcohol and disinfectant spraying can be detrimental to the mobile device. As an alternative, accessory technologies such as self-cleaning screen protectors for mobile phones have gained interest among users. These screen protectors can come in plastic films or tempered glass.

1.2.2. Embedded antibacterial screen protector coating

The increasing use of mobile phone device also correspond to the rise of mobile phone screen protectors. Initially, screen protectors are an accessory device to improve scratch-resistance and decrease direct damage of touch screens due to daily phone usage which can lead to wear and tear. This accessory can save cost from repairing of screens and can improve screen durability (Pereira et al., 2019).

Nowadays, an emerging disinfecting technology in screen protectors is added by embedding it with antimicrobial coating on them. The objective behind this innovation is to create a self-cleaning protector, made of glass or plastic film, placed where users usually touch the device. A summary of the antibacterial coatings that have been developed and tested are shown in Table 1.

Most common materials used for this technology are Titanium Dioxide (TiO_2) photocatalytic (or light-activated) coating, Copper-based Coating, Reactive Oxygen Species (ROS)-based coating, and Quaternary-Ammonium Compounds (QAC). Such coatings are embedded or applied onto the physical screen protector which may either come in plastic film or tempered glass surfaces.

Recently, industries have done patents for their production of antimicrobial screen protectors and their commercialization to consumer market. One of these companies is KASTUS (2016) who produced an antimicrobial screen protector which claims to significantly work against bacteria and viruses within two minutes. The antimicrobial activity of the screen protector is due to the titanium alkoxide and copper coating. While there have been some recent sales of "self-disinfecting" screen protectors, researchers have yet to evaluate the efficacy of microbial dissipation for TiO_2 coating on mobile phone screen protectors. Moreover, the efficacy of titanium dioxide coating on surfaces in intensive care units has been observed to have no effects on the microbial colonization on such surfaces (De Jong et al., 2018).

The QAC-based coating on glass surfaces by Saif et al. (2008) only exhibited a rapid decrease in bacterial count after 72 h. This may pose as a problem with high-touch surfaces such as mobile

devices as antibacterial activity, especially in the hospital setting, needs to be effective in a short period of time. Also, excess or deficiency of the copper-based material can be lethal and hard to clean without corrosion application (Govind et al., 2021; Ketchel, 2017).

Table 1. Summary of the Existing Antibacterial Coatings

Antibacterial Coating	Active Material	Bacteria Tested	Reference
Light-activated	Crystal violet	*E. coli*, *S. aureus*	Page et al., (2015)
Nanofixit™	Titanium dioxide (TiO_2)	---	Nanofixit Inc. (2018)
-	Copper*	*E. coli*, *S. aureus*	Sharifahmadian et al (2013)
Quaternary-Ammonium Compounds (QAC)*	Tetraethoxysilane (TEOS) and $Q4N^{+}-Si(OR)_3$	*E. coli*, *S. aureus*	Saif et al. (2008)
KASTUS Antiviral and Antibacterial Touchscreen Protector	Titanium alkoxide and copper	*E. coli*, *S. aureus*	KASTUS (2016)

*Not yet commercialized as mobile phone screen protector coating

1.3. Silver Nanoparticles (AgNPs)

Research has shown that metal nanoparticles pose excellent potential for being antibacterial coatings and can kill numerous bacteria. Some examples of these metal nanoparticles are gold (AuNPs), copper (CuNPs), Zinc (ZnNPs), and silver (AgNPs) (Sánchez-López et al., 2020; Shahzadi et al., 2018). These nanoparticles have been prominent in being an alternative to antibiotics due to the rise of bacterial resistance (Sánchez-López et al., 2020). These studies have presented promising results to the use of these nanoparticles in antibacterial activity from gram-positive and gram-negative types of bacteria.

Known for its great use in various consumer and commercial products, silver nanoparticles have been paid close attention in being an antibacterial coating agent. In comparison to other metal nanoparticles, AgNPs is more advantageous in terms of antibacterial activity and stability. With a potential use for commercial applications, AgNPs is a much-favoured metal nanoparticles as it has a higher antibacterial activity compared to the other aforementioned metal nanoparticles (Sánchez-López et al., 2020). Also, this metal nanoparticle has less oxidation aftermaths during synthesis which are observed in copper nanoparticles (Salleh et al., 2020). This can be a problem for scientists since it is difficult to obtain the desired nanoparticle due to rapid oxidation of copper to form copper oxide (CuO) which can be toxic in higher amounts.

The current advances in the study of silver nanoparticles have brought about numerous innovations that are applied to a variety of commonly used items such as medical instruments, infant products, cosmetics, and food coatings (Seltenrich, 2013). Silver nanoparticles on glass is a more novel technology. However, several studies have evaluated the embedment of AgNPs onto glass. Hofmeister et al. (2005) has noted the stability of AgNPs embedded onto glass under various techniques of fabrication. Pallavicini et al. (2010) assessed the antibacterial activity of AgNPs on glass. Nanoparticles were observed to be firmly grafted onto the glass surface even after four weeks. Furthermore, the AgNPs exhibited antibacterial activity even on the 15th day of study. An acceptable microbicidal activity against *E. coli* was achieved after 5 hours of contact with the AgNPs while that against *S. aureus* was obtained after 24 hours (Pallavicini et al., 2010a).

As a widely used nanoparticle for study (Shahzadi et al., 2018), its physical attributes such as size and stability has been vital in the improvement of antibacterial and antifungal properties.

1.3.1. Antibacterial activity of silver nanoparticles

Several evidence have revealed silver nanoparticles exhibiting antibacterial activity against a wide variety of gram-negative and gram-positive bacteria. Among the bacterial species it is effective against are *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* (Dallas et al., 2011; Vanamu et al., 2018), *Klebsiella pneumoniae* (Dallas et al., 2011), multiple antibiotic resistant (MAR) *Klebsiella pneumoniae* (Sen et al., 2013), *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Staphylococcus epidermis, Candida tropicalis*, and *Candida parapsilosis* (Dallas et al., 2011). The minimum inhibitory concentration of silver nanoparticles against Methicillin-resistant *S. aureus* is 0.350 g/L and against *K. pneumoniae* is 0.177 g/L (Dallas et al., 2011).

1.4. Synthesis of chitosan-silver nanoparticles (ChAgNPs)

The selection of a good reagent and auxiliaries are based on the criteria of conducting a process in which the reagents used are environmentally friendly and pose less toxicity on health (Iravani et al., 2014). Thus, some of the well-used materials for the reduction process are organic substances such as biopolymers, and those that can be extracted from natural resources are much favoured in the green synthesis of metal nanoparticles (Iravani et al., 2014). Unlike other chemical agents such as borohydrides and hydrazine which are detrimental to the environment and health, the use of green reagents is considered to align with the aim of reducing risk as stipulated in the principles of "green chemistry" (Anastas & Zimmerman, 2013).

1.4.1. Commonly used polymers in the reduction of AgNPs

Biological approaches have been preferred to overcome the drawbacks of chemical and physical synthesis of AgNPs which are extreme cost and use of toxic, hazardous chemicals that may pose environmental and biological hazards (Gudikandula & Maringanti, 2016). Toxic reducing reagents are often utilized in the chemical synthesis of metal nanoparticles. As an effect of the

toxicity of chemical reducing reagents, the synthesized metal nanoparticle could not be used in biomedical applications because of the existence of toxic capping agents (Phan et al., 2021).

With that, such approach has been widely researched as an alternate method for preparing silver nanoparticles. Since biologically mediated synthesis does not involve hazardous or toxic chemicals, it has been shown to be cost-effective, easy, and environmentally safe. One approach in the biologically mediated synthesis of AgNPs is the use of polymers. Polymeric material solutions contain size-confined, nanosized pools of inter- and intramolecular origin, which can be used for the synthesis of nanoparticles. The polymers used to make silver nanoparticles are listed in Table 2.

Table 2. Common biopolymers used in the synthesis of silver nanoparticles

Polymer	Silver Precursor	Purpose of Polymer	Reference(s)
Starch	$AgNO_3$	Starch acted as the stabilizing agent while β-d-Glucose acted as the green reducing agent.	Raveendran et al. (2003)
Cellulose	$AgNO_3$	Cellulose nanocrystals were used as the reducing agent to synthesize the silver nanoparticles.	Drogat et al. (2011)
Carboxymethylated-curdlan	$AgNO_3$	CM-curdlan functioned as the stabilizing agent in the synthesis of silver nanoparticles.	Leung et al. (2010)
Fucoidan	$AgNO_3$	Fucoidan acted as a stabilizing agent	Leung et al. (2010)
Gelatine Protein	$AgNO_3$	Nano-scale silver was stabilized using gelatine protein in the presence of $NaBH_4$	Halder et al. (2011)
Chitosan	$AgNO_3$	Chitosan function as reducing and capping agent in the synthesis of AgNPs.	Nate et al. (2018) Phan et al. (2021)
Glucan	$AgNO_3$	Glucan functions as stabilizing agent in the synthesis of AgNPs	(Sen et al., 2013)
Bombyxmori Silk Fibroin	$AgNO_3$	*Bombyxmori* Silk Fibroin functioned as reducing agent in the *in-situ* synthesis of silver nanoparticles.	Shivananda et al. (2016)

Chitosan, with chemical structure shown in Figure 1, is a natural polysaccharide with known biodegradable and biocompatible characteristics, is often used for the reduction of metal nanoparticles. This polysaccharide does not introduce environmental toxicity and biological hazards (Huang & Yang, 2004). Several studies have also reported that chitosan is an effective reducing reagent in the synthesis of silver and gold nanoparticles (Huang & Yang, 2004; Nate et al., 2018; Phan et al., 2021). In the presence of chitosan, the reduction of Ag^{+} ions to Ag^{0} occurs even at room temperature owing to the free electron provided by the oxidation of alcohol or glucosidic groups in the chitosan molecule. In connection, when the reaction temperature is increased, the reduction reaction occurs faster (Phan et al., 2021).

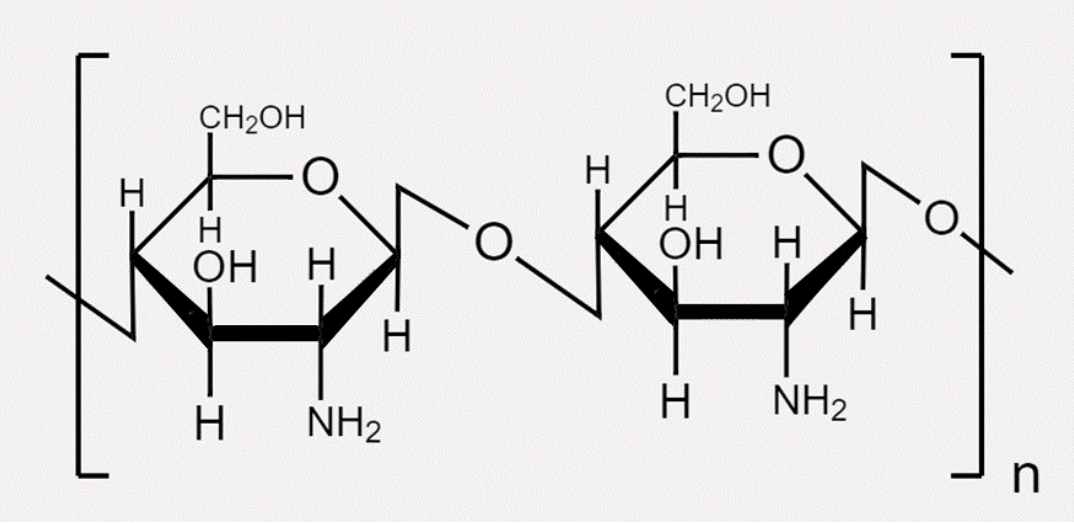

Figure 1. Chemical Structure of Chitosan

Many researches have conducted laboratory scale experiments entailing the formulation of Chitosan-silver nanocomposites (ChAgNPs) and its usage in food coatings (Nisha et al., 2016; Salgado-Cruz et al., 2021), antimicrobial coatings (Moodley et al., 2018; Qi et al., 2004; Regiel et al., 2013), packaging (Kumar et al., 2020), and other applications.

Among the listed polymers that are employed in the synthesis of silver nanoparticles, chitosan is one of the polymers that could both function as reducing and capping agent. Due to this capability of chitosan, it would be utilized as the capping and reducing agent in this study.

1.4.2. Reduction and stabilization of AgNPs

The mechanism of the reduction of Ag^{+} to Ag^{0} is kinetically carried out by electron transfer from the metal ion to its reducing agent. This can be made possible under elevated temperature but may also occur under room temperature (Grumezescu, 2016)

$$AgNO_3 \rightarrow Ag^{2+} + NO_3^{-} \quad (1)$$

$$Ag^{+} \rightarrow Ag^{0} \quad (2)$$

Chitosan can be used as a multifunctional template which acts as a reducing agent and a stabilizing agent in the synthesis of the chitosan-silver nanocomposite. In some studies, they have concluded the possibility of carbohydrate and amino group polymers to show positive results of being a reducing agent and stabilizing agent for metal nanoparticles (Rahman et al., 2018; Rao & Pennathur, 2017; Vanamu et al., 2018). Furthermore, this polysaccharide is a good stabilizer for metal nanoparticles because of its availability, biocompatibility, and highly positive charge. As depicted in Figure 2.a, metal nanoparticles agglomerate in solution because of Van der Waals interactions between the metal surfaces. On the other hand, Figure 2.b, the chitosan creates a stearic barrier with a positive charge density covering the metal core. Furthermore, the formation of homogeneous metal nanoparticle solutions, as shown in Figure 2.b, is attributed to the strong electrostatic interaction among the positively charged metal cores (Phan et al., 2021).

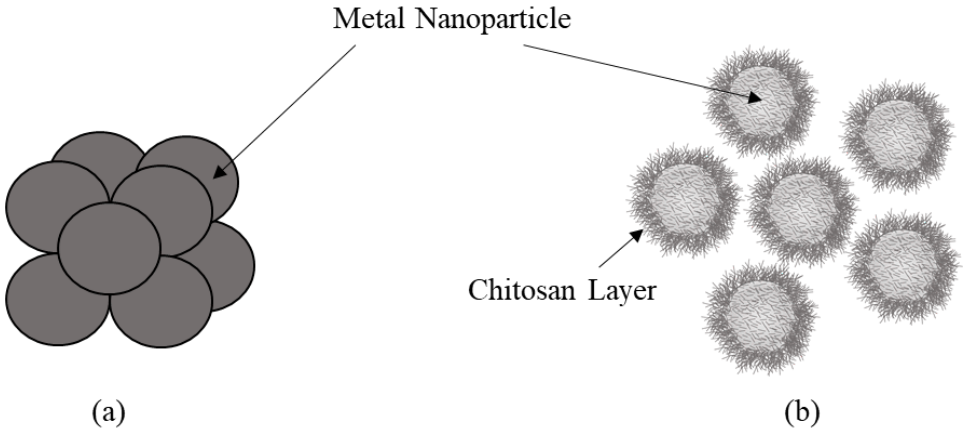

Figure 2. The effect of chitosan on the metal nanoparticle dispersion. (a) The aggregation of metal nanoparticles without chitosan (b) The metal nanoparticle dispersion with added chitosan (adapted from Phan et al., 2021)

1.4.3. Factors affecting the synthesis of ChAgNPs

Like any other chemical reaction, several factors affect the bottom-up synthesis of chitosan-silver nanocomposites. In the subsections, the roles of pH, reaction temperature, reaction time, and the reduction and capping agent on the synthesis process will be discussed.

A. pH

The pH level is an indicator used to specify the acidity or basicity of an aqueous solution. Acidic solutions tend to have a lower pH than those measured on basic or alkaline solutions. In the synthesis of ChAgNPs, pH played a major role on the distribution on the hydroxyl and amine groups which in turn affects the size of the nanoparticles (Sibiya & Moloto, 2014).

At lower pH, chitosan carries a positive charge because of the protonated amino group, thus, allowing it to interact with negatively charged species (Nate et al., 2018). On the other hand, the addition of a base allows a basic environment that eventually conducts deprotonation in the amino group of the chitosan molecule. The deprotonation leaves a negative site on the amine group, which causes it to have a high affinity towards positive metal ions, in this case, silver ions. The reduction of Ag^{+} to Ag^{0} follows the silver ion binds to the chitosan molecule, which then generates the nanoparticle, wherein the Chitosan serves as the shell of the core silver nanoparticle.

The addition of Sodium Hydroxide (NaOH) is known for its large impact on the reaction kinetics of particle formation, and is the key factor in regulating the mono-dispersity of the synthesized AgNPs (Nishimura et al., 2011). Furthermore, the addition of NaOH accelerates the reduction reaction of Ag^{+} to Ag^{0} and the nucleation of AgNPs. The increased reduction and nucleation rates, as proposed by Nishimura et al. (2011), is due to the formation of silver oxides (Ag_2O) intermediate in the presence of NaOH. The formation of Ag_2O was also observed under room temperature conditions. Moreover, the Ag_2O is then converted silver hydroxide, Ag(OH), after ramping up the temperature followed by the formation of AgNPs. The reduction rate of Ag(OH) was also observed to be higher than silver nitrate ($AgNO_3$) even under the same reaction conditions. In connection, the addition of NaOH increases the pH thus increasing the ability of reducing agents such as chitosan. Consequently, the reduction reaction kinetics are affected synergistically and the formation rate of AgNPs are dramatically increased with increasing NaOH concentration (Nishimura et al., 2011).

B. Temperature and reaction time

The reaction temperature is one of the key factors that influence chemical reactions. A study conducted by Jiang et al. (2011) on the temperature and growth size correlation of silver nanoparticles showed that the size of silver nanoparticle (spheres) is not linearly dependent on temperature. As depicted in Figure 3, a growth jump from 25 to 48 nm was observed on Ag nanoparticle spheres when temperature was increased from 17 to 32 ^{0}C. Further, at temperatures higher then 32^{0}C, the particle size of the Ag nanospheres decreases. These Ag nanospheres are not expected to form together with Ag nanoplates especially in the presence of a reducing agent with an amine functional group such as chitosan. Therefore, with chitosan as the reducing agent and capping agent in the synthesis of silver nanoparticles, only Ag nanospheres are expected to form.

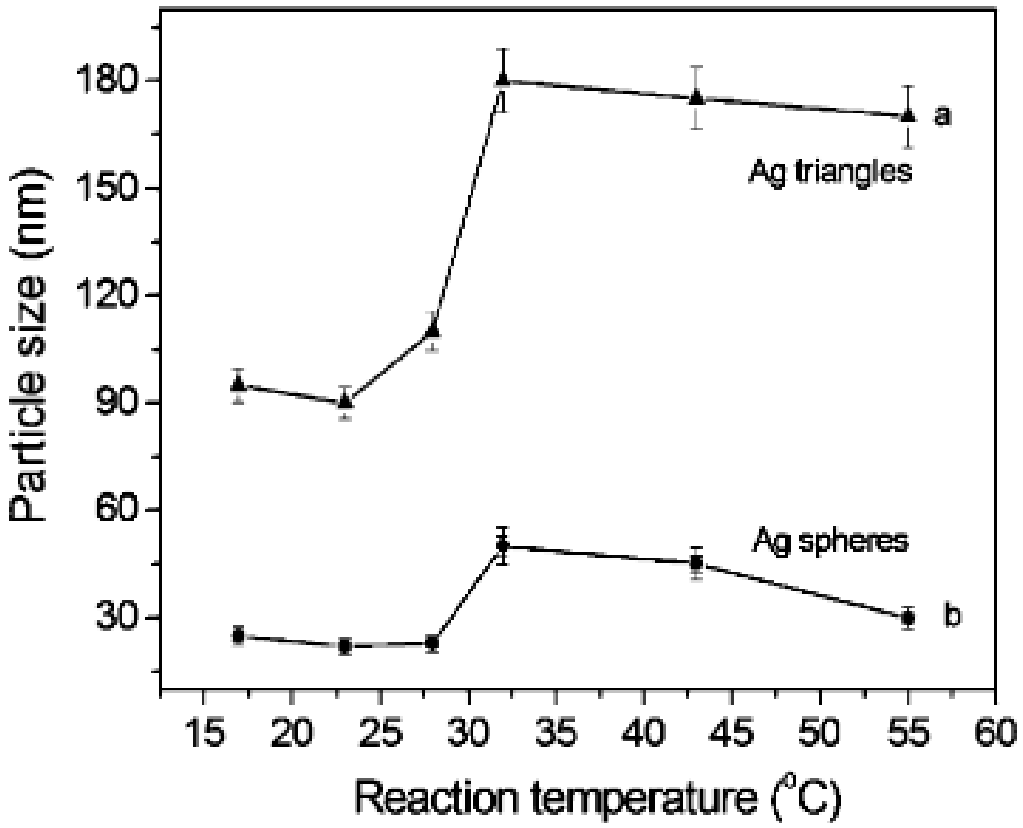

Figure 3. The average size of silver nanoplates (curve a) and nanospheres (curve b) obtained at different temperatures from 17 to 55°C (Jiang et al., 2011)

Meanwhile, a study by Maragoni (2012) focused on the ChAgNPs synthesis at varying chitosan and silver nitrate precursor concentrations. The study identified the reaction time by synthesizing chitosan and silver nitrate at equal composition (0.5% w/v) at varying reaction time from 10 through 60 minutes and are studied by obtaining a UV-visible absorption spectra. These spectra are presented in Figure 4. It was reported that the reaction was efficient by increasing the reaction time. Such study utilized 50 minutes reaction time and was able to obtain positive results in their study.

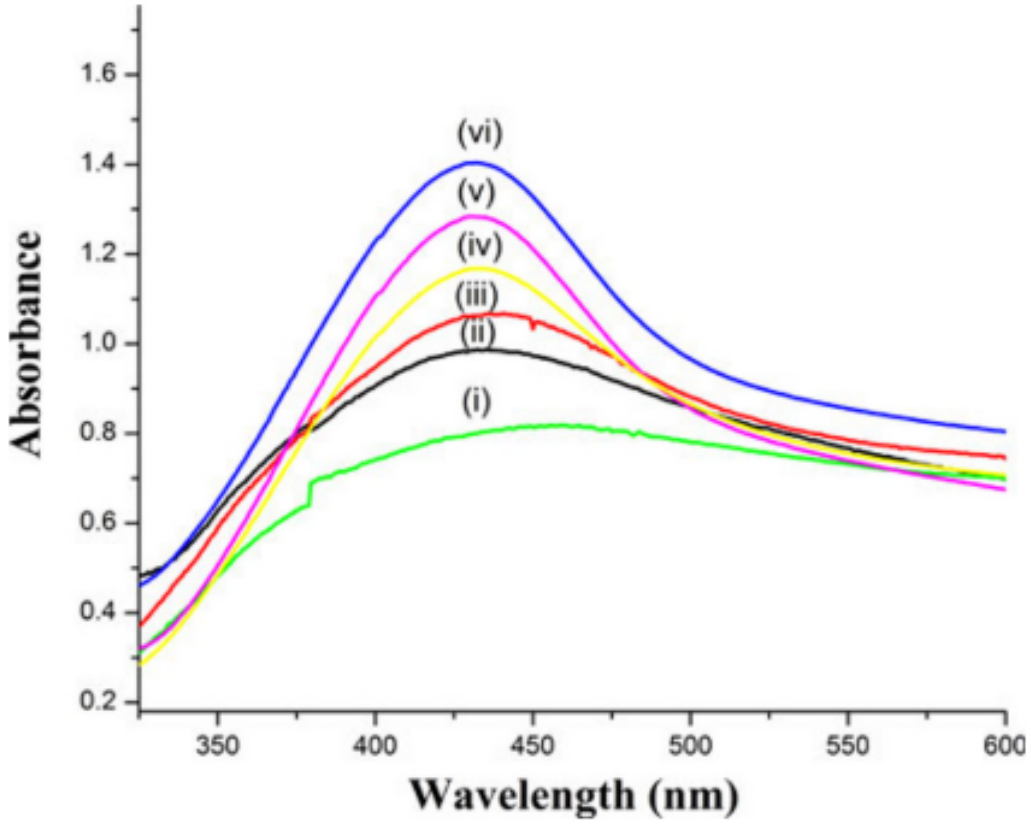

Figure 4. UV-visible spectra of the ChAgNPs at varying reaction time; (i) 10 minutes, (ii) 20 minutes, (iii) 30 minutes, (iv) 40 minutes, (v) 50 minutes, and (vi) 60 minutes (Maragoni et al., 2012)

C. Size and shape

Nanoparticles are classified as a 0D nanomaterial. This type of materials has all its dimensions within the 1-100 nm range (Mohamed, 2017). This can be identified with the use of electron microscopes (Nouailhat, 2010). Moreover, the reduction method of the metal nanoparticles falls upon a bottom-up approach where a self-assembly of species occurs along with size-controlling agents (i.e., capping agents). This approach is mostly done under a fluid phase since the creation of nanomaterials are done at an atomic or molecular dimension (Elnashaie et al., 2015).

Chitosan functions as a size control agent in the synthesis of metal nanoparticles. According to Phan et al. (2021), the sizes of the synthesized nanoparticle was decreased with the addition of chitosan at higher concentrations. The correlation between the chitosan concentration and the

size of the metal nanoparticle, shown in Figure 5 was also observed in the synthesis of palladium and gold nanoparticles. Phan et al. (2021) proposed a hypothesis to explain how the concentration of chitosan affects the size of the metal nanoparticles. The hypothesis stated that in the formation process of the metal nanoparticles in the presence of chitosan, the positively charged chitosan strongly interacts with the metal nuclei. Thus, the higher chitosan concentration leads to stronger chitosan interaction with the metal nuclei. Furthermore, the strong interaction between the metal nuclei and chitosan inhibits the binding precursors to the metal nuclei, resulting to the inhibition of metal nuclei growth.

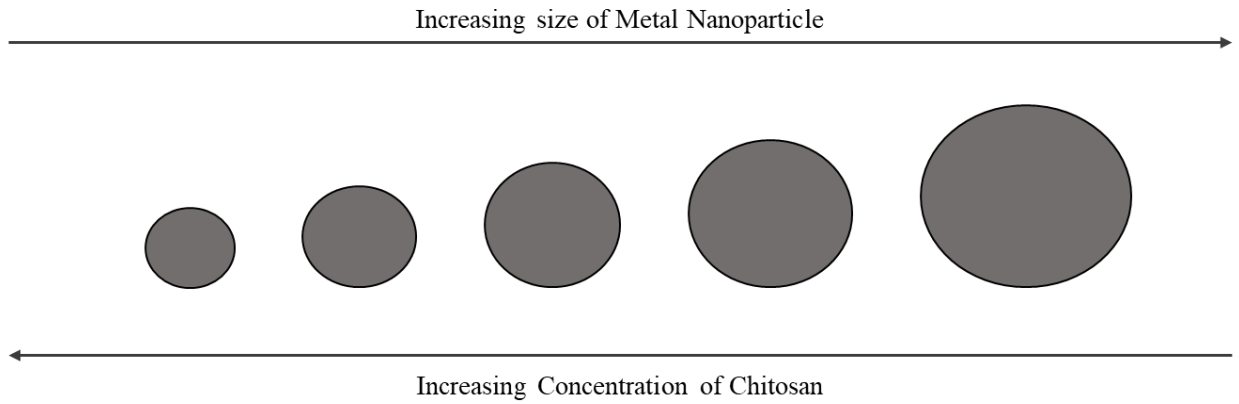

Figure 5. The Correlation of Concentration of Chitosan and The Size of Metal Nanoparticles (adapted from (Phan et al., 2021))

When added to the reaction solution, chitosan causes either an electrostatic interaction between the positively charged Chitosan and the negatively charged silver nanoparticles or the absorption of Chitosan onto the silver nanoparticle surface, forming a core-and-shell nanoparticle. This mechanism is necessary to ensure the "nano" size and shape of the nanoparticles, which has a significant effect on their properties (Phan et al., 2021).

Many studies have reported that a spherically shaped core-shell structure is observed upon synthesis of ChAgNPs despite its varying methods and reagents used. Its size can range from 20 to 50 nm where aggregated AgNPs are surrounded by chitosan which controls the metal nanoparticle's size (Alshehri et al., 2020; Botelho et al., 2021; Nguyen et al., 2013). It is further reported by Jiang et al. (2011) that the particle size of AgNPs are smaller for spherical types than

that of triangular nanoplates. This is further desired in studies for antimicrobial tests as smaller nanoparticles yield a more efficient antiviral activity (Dallas et al., 2011).

A limited number of studies have been carried out in observing the relationship between chitosan concentration and AgNP size. One of which is that from Nate, et al. (2018) where the synthesis of ChAgNPs occurs at 0.5, 1.0, and 2.0 %w/v chitosan concentration. It was observed through TEM that the smallest average nanoparticle size was measured 2 nm, using 2.0 %w/v of chitosan concentration. Another one is from Margoni et al. (2012). They observed in a UV-vis spectrophotometer (see Figure 4) that the absorbance spectra at around 420 nm wavelength increases as the concentration of chitosan also increases. They further mentioned in their findings that this is indicative of an increased efficiency in the nanoparticle synthesis. However, these studies differ in terms of ChAgNP synthesis and of how they examined their samples. This research aims to introduce a more facile approach in the synthesis of ChAgNPs and to clearly observe the relationship between chitosan concentration and the nanoparticle size by measuring the average size in each produced sample.

1.5. Characterization of ChAgNPs

The importance of a characterization procedure in nanomaterial synthesis is to verify the success of the process and that the produced substance would fall within the nano-scale range (Nouailhat, 2010). The verification of a successful nanoparticle formation is examined through imaging and spectrum data which corresponds to the presence of the nanocomposite and its stability to maintain its state and form. The following subsections are some of the common characterization measures that are used to identify nanomaterials at various scales and types.

1.5.1. Stability (Zeta-Potential)

To assess the surface charge and stability of the nanocomposite, zeta-potential is measured in a zeta-sizer. Based on a study by Selvamani (2019), zeta potential is a quantification of the effective electric charge that surrounds the surface of the nanocomposite. It measures the difference between the oppositely charged ions on the nanocomposite surface and the bulk fluid

where the dispersion of nanocomposite takes place. This can play a huge role on the stability behaviour and toxicity of the nanomaterial (Lowry et al., 2014).

The principle behind zeta potential is based on the presence of an electric potential difference caused by the interaction between the nanoparticle solid and bulk fluid (i.e., the liquid solvent). A surface charge is produced from the interaction such that the surface ions of the nanoparticle tend to attract oppositely charged ions in the bulk medium (Barn, 2020).

When an electric field is introduced to the colloid solution, an electrokinetic phenomenon occurs wherein particles move within the interphase region. This phenomenon is also called electrophoresis. Potential, from a known external electric field, is measured which is then a function of the particle velocity caused by a Doppler shift or a change in wave frequency relative to its source. The frequency shift is obtained to indirectly determine the electrophoretic mobility or velocity of the nanoparticle (Hunter, 1981). The frequency shift of system also determined the speed of particle motion which then determines the amplitude of the electric charge. Such measure abides ISO 13099 of 2012 which explains in full detail the measurement of zeta potential.

The result produced in this characterization will be a plot of particle velocity as a function of either the hydrodynamic diameter with respect to the surface of the nanoparticle or the logarithmic scale of H^+ concentration or pH. In ISO 13099:2012 standards, the stability of a metal core nanoparticle is set to be more than 40 mV regardless of being positive or negative in charge. For plots with abscissa of pH, the x-intercept, or the pH value at which the potential is recorded zero, is determined to be the isoelectric point of the colloidal system.

Such measurement is understood by having a good grasp on the suspension of nanomaterials in a fluid. There are two distinct layers that can be found within the suspended material which are the strongly bonded layer called the stern layer and the loosely bonded layer called the diffused layer. A diffusion of ions is observed across these two layers in which the ions would cling through the nanoparticle. An observed boundary layer from the diffused layer to the bulk fluid

called the slipping plane where the particle would interact to other surfaces (Lowry et al., 2014). This slipping plane is the point at which the zeta-potential is measured, and stability is assessed.

1.5.2. Fourier Transform Infrared Spectroscopy (FTIR)

The purpose of the FTIR is to confirm the identity of the nanocomposite by determining the presence of molecular bonds in the formation of the nanocomposite. This identifies a molecular "signature" which can indicate the presence of the substance within the sample system. This "signature" corresponds to the absorption peaks which can be measured through the device (Dutta, 2017) and may also indicate the amount of material present by observing the size of spectrum peaks (Ganzoury et al., 2015).

The principle behind the FTIR is based on the infrared photons that interact with the sample, causing vibration between chemical bonds. These bonds have specific frequencies, which is absorbed by infrared (IR) rays. The obtained signal from the outgoing IR beam is translated through a spectrum, which would identify the functional groups and compounds present. An interferometer is also used to measure the wavelength of interference patterns, which helps in the simultaneous measurement of IR frequencies. From the data obtain from the interferogram, the Fourier Transform mathematical process translates the frequencies and produce a spectrum plot of transmittance (T) against wavenumber – or the reciprocal of wavelength (Govindan et al., 2012; Sindhu et al., 2015).

The signature FTIR spectrum of ChAgNPs is observed from studies by Ali et al. (2011) and Margoni et al. (2012). Based on the spectrum plot presented in Figure 6, significant bends and stretches are observed which corresponds to a specific bond that is present in the nanocomposite. At around 3429 cm^{-1}, a peak is observed which represents the presence -NH_2 and -OH groups found in chitosan. The peak at 2881 cm^{-1} being lower for ChAgNPs can be caused by the reduction and stabilization process where primary amine ions are transferred. The same is observed in wavenumber 1657 and 1600 cm^{-1} in the chitosan spectrum which represents -$CONH_2$ and -NH_2 groups. Upon reduction to ChAgNPs, the disappearance of these two peaks is observed, and a new band appears at 1635 cm^{-1} which indicates the attachment of reduced silver.

Compared to pure chitosan, the peak intensities at wavenumber range of 1000-1350 cm^{-1} are lower due to the complexation of chitosan with silver (Ali et al., 2011).

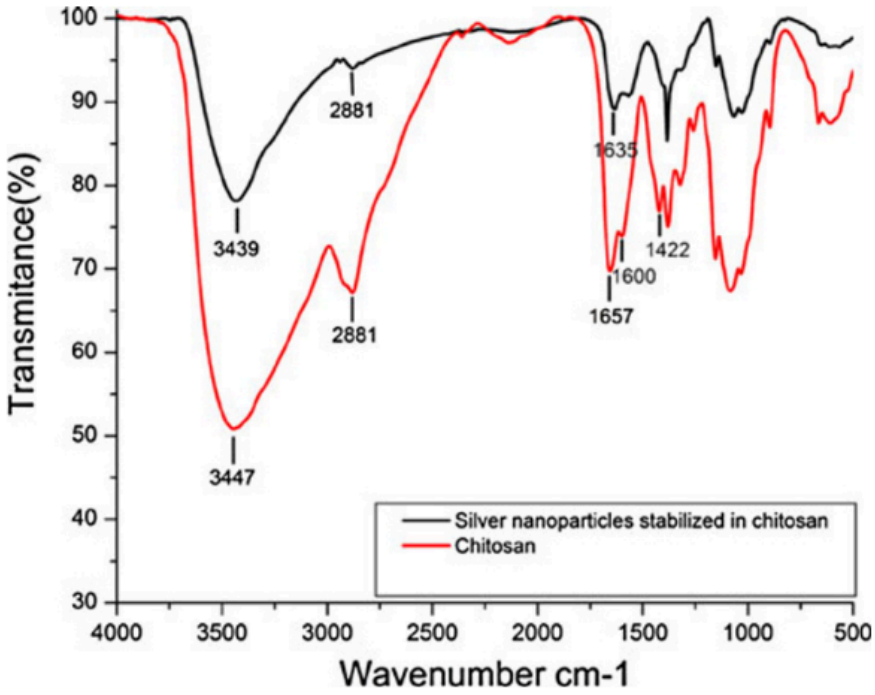

Figure 6. FTIR spectrum for chitosan (in red) and ChAgNPs (in black) (Maragoni et al., 2012)

1.5.3. Field Emission Scanning Electron Microscopy

The Field Emission Scanning Electron Microscope (FESEM) is a morphological characterization equipment that contains larger magnification levels to observe objects on micro- to nano-scale images. It differs with SEM such that a field emission source is used for electron beam production which is 1000 times smaller than the latter (Elnashaie et al., 2015). Although, the principle is similar where secondary electrons are detected upon contact between the sample and the electron beam (Sharma et al., 2018). Also, FESEM yields a clearer image of the sample through an additional topographical contrast at very low voltage requirements. It also has an energy-dispersive X-ray spectroscopy (EDX), which detects elemental substances, where it may also examine minor contamination spots within the sample (Mayeen et al., 2018).

1.6. Coating of ChAgNPs onto tempered glass screen protectors

One of the most used types of screen protector for mobile phones are tempered-glass types due to their durability, scratch-resistance, and anti-fingerprint technology embedded in them. With these properties, such type of screen protector has seen rising sales in the market. In the findings from a recent study by Reports and Data (2020), much of the demand in the global market screen protectors come from those made of glass material compared to other raw materials such as polyethylene terephthalate (PET) and Thermoplastic Polyurethane (TPU). It can be deduced that tempered glass protectors exhibit great demand in the market nowadays and will continue to do so
in the future.

However, due to immense usage of mobile phones with screen protectors in daily activities in many settings, the probability of fomite transmission of bacteria is greater especially when these come from nosocomial types. Thus, this study is conducting an application process of a coating material – i.e., green synthesized ChAgNPs – onto screen protectors for an added antibacterial protective layer.

To assert that ChAgNPs can coat the surface of tempered glass, it is best to identify how chitosan can potentially adhere to the glass surface. The concept of adhesion of nanoparticles to glass surfaces is suggested to be caused by molecular bonding which is widely accepted in several studies in terms of the adhesion of two surfaces (Awaja et al., 2009). Such close contact between the two surfaces entails several intermolecular and intramolecular forces that act to strengthen its adhesion.

The adhesion of nanoparticles occurs either through adhesive adhesion or particle adhesion. According to Joo & Baldwin (2010), the adhesive adhesion theory states that two surfaces, upon close contact, have mechanical interlocking and physical adsorption where intermolecular forces such as van der Waals interaction between parallel surface and chemicals bonds take place. An analogy to this theory is a glue-on-wood concept where substrates stick to each other despite rough irregularities (Awaja et al., 2009). On the other hand, particle adhesion also has Van der Waals and chemical bonds as a conceptual mechanism of interaction, but such interaction occurs

between a flat surface (glass) and spherical particle (ChAgNPs). These long-range and short-range forces where the intermolecular and intramolecular bonds establish possibility of the adhesion contact area between the nanoparticle and glass substrate.

In the case of AgNPs, a study by Schneid et al (2015) has presented a successful AgNP coating process unto glass surface using ionic sesquioxide as stabilizing agent. The nanoparticle was uniformly distributed unto the glass slide surface through dip-coating technique and thermal treatment. It also reports that, at room temperature treatment, agglomeration of AgNPs is minimal with results showing an average size measure of 2.4 nm. Thus, it is possible to coat AgNPs with stabilizing agents such as chitosan unto glass surfaces. On the other hand, to ensure adhesion of chitosan on glass surfaces, a physical curing method can be administered (Vieira et al., 2014; Wan et al., 2010). In the physical curing method, the chitosan-coated glass can be oven dried at 50^0C for 12 hours.

In this study, the application of chitosan-silver nanocomposite as an antibacterial coating on tempered glass screen protectors will be facilitated using the drop-casting method. This method is a simple and low-cost deposition method for film coating. Furthermore, unlike spray coating and dip-coating techniques, the drop-casting method is a facile and a more-controllable coating technique. In addition, controlling the repeatability and uniformity of the spray-on films is reported to be difficult. According to Eslamian & Soltani-Kordshuli, (2018), if the coating application is limited to a small-area thin solid film, the drop-casting method is the most appropriate. Drop-casting depends on the release of large droplets that spread and wet the surface upon impact (Bormashenko et al., 2019; Eslamian & Soltani-Kordshuli, 2018). Chandren & Zulfemi (2019) suggested that to produce a uniform layer of coating on a film of approximately 6 cm^2 in area, 0.2 mL of the nanoparticle solution should be deposited on the film using a 1-mL micropipette. Different studies have also reported the effectiveness of the drop-casting technique in the polymer coating application on glass substrates (Cheng et al., 2018; Esteves et al., 2014; Melavanki et al., 2020).

1.7. Antibacterial property of ChAgNPs

Chitosan-silver nanoparticles showed effective antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Badawy et al., 2019; López-Carballo et al., 2013; Paulkumar et al., 2017; Raghavendra et al., 2016; Wongpreecha et al., 2018), *Bacillus subtilis*, *Streptococcus faecalis, Pseudomonas aeruginosa* (Paulkumar et al., 2017), and *Staphylococcus epidermis* (Kolarova et al., 2015) and mosquitocidal properties (Alshehri et al., 2020). This material has also exhibited good potential in applications such as food preservation as active and intelligent packaging (Qin et al., 2019) and as a coating for fruits and vegetables (Ragunathan et al., 2015) and in biomedical applications as a wound dressing (Kolarova et al., 2015).

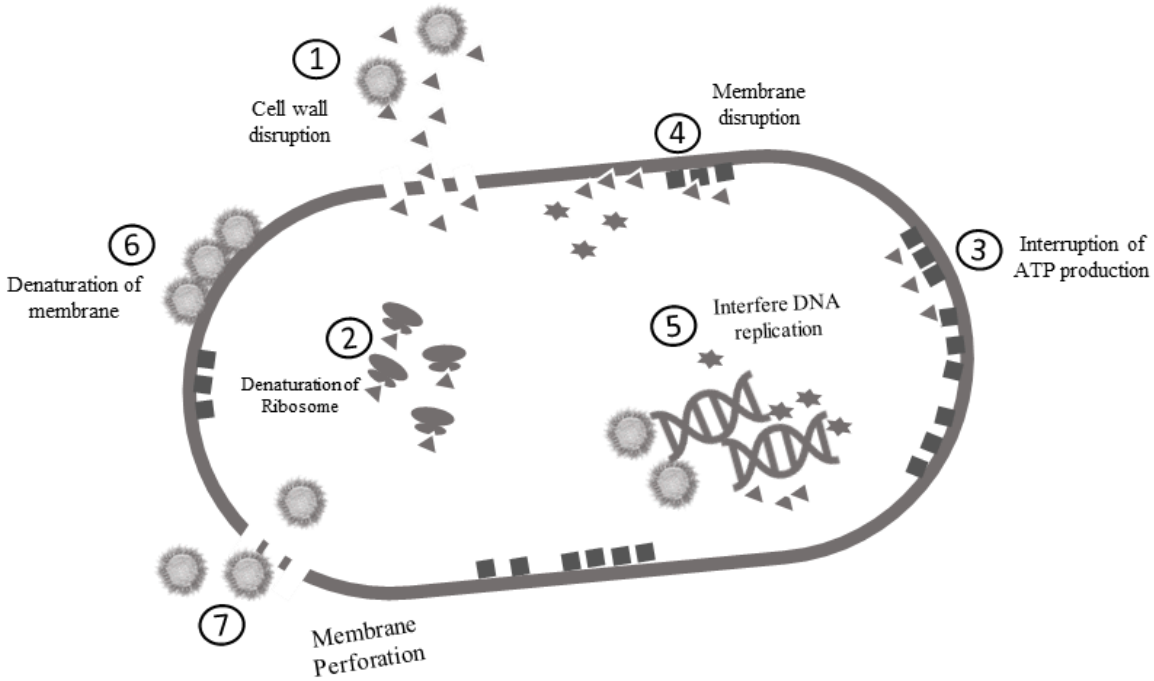

Figure 7. Proposed Antibacterial Mechanisms of Chitosan-Reduced Silver Nanoparticles (adapted from Yin et al., 2020)

Figure 6 illustrates some of the mechanisms of the antibacterial activity of the nanoparticles as proposed by Yin et al. (2020). AgNPs constantly deposit silver ions onto the cell wall, and these ions adhere there and to the cytoplasmic membrane. The ions then alter the permeability of the membrane, resulting to the disruption of the bacterial envelope as in (1). They can also cause the denaturation of ribosomes in the cytoplasm as in (2). In (3), adenosine triphosphate (ATP)

production is halted as reactive oxygen species (ROS) is generated (4). The sulphur and phosphorus in deoxyribonucleic acid (DNA) react with the silver ions, interfering with DNA replication and cell reproduction (5). This may even cause the death of the microorganism itself. As shown in (6) and (7), AgNPs may also be instrumental in the denaturation or in the perforation of the cell membrane in earlier steps.

A summary of the antibacterial activity results of ChAgNPs in previous studies, including the proposed or observed antibacterial mechanisms, are shown in Table 3.

Table 3. Summary of the studies on the antimicrobial mechanism of ChAgNPs

Reference	Bacteria Tested	Mechanism of Antibacterial Action
M. E. I. Badawy et al. (2019)	*E. coli, S. aureus*	● Inhibition of cell replication
López-Carballo et al. (2013)	*E. coli, S. aureus*	● Adhesion of AgNPs to the cell membrane, changing its permeability and attacking the respiratory chain of bacterial cell* ● Condensation of the DNA molecule, suppressing bacterial cell replication*
Paulkumar et al. (2017)	*E. coli, S. aureus, B. subtilis, S. faecalis, P. aeruginosa*	● Electrostatic interaction between deposited positively charged silver metal ions with the negatively charged DNA and protein molecules, causing the collapse of cell structure and function
Raghavendra et al. (2016)	*E. coli, S. aureus*	● Inhibition by formation of pits, leading to microbial death
Wongpreecha et al. (2018)	*E. coli, S. aureus*	● Disruption of cell wall, leading to impeding of DNA replication and denaturation of proteins

Kolarova et al. (2015)	*E. coli, S. epidermis*	(No explicit mechanism described)

**Mechanism observed in the study*

In terms of the relationship of nanoparticle and antibacterial activity, research have shown inverse relationship where better antibacterial effects are observed with the use of very small nanoparticle size. As the concept of decreasing the particle size of substance is an observed change in its physical and chemical properties, the observation of an improved antibacterial property was also found to show positive outcomes especially from metal nanoparticles (Martínez-Castañon et al., 2008).

Gram-positive bacteria have a cytoplasmic membrane and cell wall of thickness 20-28 nm. Meanwhile, gram-negative bacteria have two-cell membranes with a thickness of roughly 7-8. In order to strike through the nucleus of the bacteria, a substance as small as its thickness is required to pass through and would susceptibly damage the bacteria cell, causing elimination or death of the microorganism (Sirelkhatim et al., 2015). Thus, it is a crucial factor to determine the size of the nanoparticle through the synthesis process and its factor that affect such reaction.

Some proofs of such relationship were evident in the experiment done by Raghupathi et al. (2011) on zinc-oxide nanoparticles (ZnONPs). It was observed that the recovered viable cells increased as particle size increased which regard that inhibition is significantly observed at very small nanoparticle size. The same observation was observed in the study conducted by Martìnez-Castañón. et al (2008) for AgNPs with gallic acid as its reducing agent. Result showed that at 7-nm diameter, a very low minimum inhibition was observed for *E. coli* and *S. aureus* bacteria. These studies prove that the smallest possible nanoparticle made in the synthesis is the optimal candidate for antibacterial activity and shall be used for assessment during application.

However, there was no study done yet on the factor of contact time as variable to the antibacterial activity of ChAgNPs, apart from its ability to disinfect tempered glass surfaces. This factor is very important in terms such that contact time has a significant effect on the efficacy of the material being an antibacterial agent (West et al., 2018).

Henceforth, based on the literature studies and determined knowledge gaps, this study then aims to: (1) to identify experimentally the particle size trend through varying the chitosan

concentration via a "green synthesis" reduction method using a multifunctional agent (i.e., Chitosan) as reducing and stabilizing agent to of silver nanoparticles and, and (2) assess the potential of the optimal nanocomposite, whose nanoparticle size is the smallest that falls within the 0D size range of 1 nm to 100 nm, as antibacterial coating onto mobile phone glass protectors against *S. aureus* and *E. coli* based on contact time variable.

II. Significance of the Research Work

For the past several years, fomite transmission of disease-causing bacteria as a cause for nosocomial infections has become increasingly relevant. This is most especially due to the gaining popularity of mobile phones, whose screen is a high-touch surface, in people's personal and professional lives including those of healthcare workers. Thus, there is a need for mobile phone screens to have an added antibacterial function.

The synthesis of ChAgNPs supports the United Nations' 12th Sustainable Development Goal (SDG) in the promotion of responsible production as it utilizes the waste material Chitosan as a reducing and stabilizing agent for silver and does not involve toxic reagents. This is in contrast to other methods of synthesis that use citrate, borohydride, thioglycerol, and 2-mercaptoethanol which are hazardous (Zhang et al., 2016). Thus, the development of the synthesis technique for the nanocomposites promotes the usage and development of "green" technologies and products. Additionally, the results of this study will contribute significant information to the promotion of good health linked to the UN's 3rd SDG: Good Health and Well-being.

Chitosan, silver nanoparticles, and chitosan-silver nanocomposites have all shown antibacterial properties against a wide range of bacteria in various applications. However, to date, relatively little is known of the antibacterial activity of ChAgNPs as a coating material for glass surfaces, and even less is known of the time it takes for them to effectively kill bacteria. Thus, the results of this study will fill such knowledge gap and may even be essential to the development of a more effective yet "green" antibacterial mobile phone screen protector coating.

Furthermore, this study will be able to provide additional information about the ability of chitosan in capping and reducing the size of the metal nanoparticles. This information can be vital in future related studies.

III. Scope and Limitations

The synthesis of ChAgNPs in this research focuses mainly on the effects of varying the concentration of prepared chitosan, as a reducing and stabilizing agent, on silver nanoparticle size. The chitosan to be used and its corresponding degree of deacetylation will be that which is commercially available. The experimental data shall use five chitosan concentrations which are 1.0%, 1.5%, 2.0%, 2.5% and 3.0% w/v at constant $AgNO_3$ concentration, temperature, pH, and reaction time. A strandline of the average nanoparticle size, observed through a Field Emission Scanning Electron Microscope (FESEM), against chitosan concentration will be reported. A characterization of the ChAgNPs at optimal chitosan concentration (i.e., smallest particle size which falls within the range of 1nm to 100nm) will be done to determine the stability of the nanocomposite solution through its zeta potential and chemical composition through Fourier-transform Infrared (FTIR) spectroscopy.

Only the optimized ChAgNPs coating (smallest particle size) on glass panels will be investigated in the antibacterial assay. The research also shall only consider the gram-positive *Staphylococcus aureus* and the gram-negative *Escherichia coli* as the test bacteria as they are two of the disease-causing bacteria that have had evidence of fomite transmission especially in the hospital setting. The contact times between the material and the bacterial inoculum will be limited to four variants (1 min, 1 hr, 2 hr, and 24 h). The antibacterial activity results will only be based on the reduction of the number of bacteria after contact with the ChAgNP-coated glass surface (i.e., the logarithmic difference between the bacterial count between the untreated surface and the coated surface).

IV. Research Design & Methods

4.1. Materials

Silver Nitrate ($AgNO_3$) solids (ACS reagent) with 99.0% purity will be used as precursor material for the synthesis. Meanwhile, chitosan powder will be purchased from Sigma-Aldrich with a degree of deacetylation of ≥75%. Glacial acetic acid (99.7% purity) is used as solvent for the preparation of chitosan solution. For the alkalinity of synthesized nanocomposite, anhydrous, reagent grade sodium hydroxide (NaOH; 98% purity) is used.

Tempered glass screen protectors will be purchased from MIYI accessories. The type of the screen protector to be used will be a camera lens glass screen protector with dimensions 2.5cm x 2.5cm. Camera lens glass protectors will be used instead of the phone screen protectors as they are easier to handle because of their smaller size. Furthermore, both are made of the same tempered glass material.

The bacterial cultures that will be used for the antibacterial assay will be those of *S. aureus* and *E. coli*. These will be grown in Mueller-Hinton broth and agar. Saline solution (0.9% w/v sodium chloride in distilled water) will be used as dilution and recovery medium during the assay. These materials will be provided by the University of San Carlos – Biology Department.

4.2. Research framework

The aim of this study is to investigate the effects of chitosan concentration to the nanoparticle size of ChAgNPs upon synthesis and to assess the antibacterial activity of the coating containing the optimal ChAgNP sample against *E. coli* and *S. aureus* based on contact time. The research design is developed in such that a novel application of ChAgNPs, specifically on to glass surfaces, will be explored. Figure 8 presents the overall methodological framework of the study.

To meet the first objective, the synthesis of ChAgNPs will be made at varying concentration of chitosan solution and at constant amount of AgNPs – from precursor $AgNO_3$. Through a Field Emission Scanning Electron Microscope (FESEM), the average size from core-shelled ChAgNPs will be quantified using Image J, an imaging software. Based on the obtained quantitative data, a data plot is constructed with nanoparticle size against chitosan concentration. The optimal size –

i.e., the smallest ChAgNPs that will be produced in the synthesis – will be further characterized using Fourier Transfer Infrared (FTIR) spectroscopy and zeta potential. Consequently, it will be applied as a coating using drop-casting method to tempered-glass screen protectors. The antibacterial activity of the coating will also be investigated against *S. aureus* and *E. coli*. A summary of input and response variables is presented in Table 4.

Table 4. Summary of quantitative and qualitative response data of the research study.

Equipment/Assay	Input Variable	Response Variable	
		Quantitative	**Qualitative**
FESEM	Sample ChAgNPs at varying chitosan concentration	Average Nanocomposite Size	FESEM image of produced ChAgNPs
FTIR	ChAgNP at optimal* chitosan concentration	---	IR spectra of ChAgNPs (Peak Points at certain wavelengths)
Stability (Zeta Potential) Test	ChAgNP at optimal* chitosan concentration	Plot of Potential (Voltage) against Hydrodynamic Diameter	---
Measurement of Coating Thickness	Coated glass protector with ChAgNP at optimal* chitosan concentration	Thickness of coating	Colour of coated glass protector, Uniformity of coating material
Antibacterial Assay	Contact time with coating at optimal* chitosan concentration	Log reduction of the colony forming units	---

*Optimal means the chitosan concentration that will yield the smallest average nanocomposite size and will fall within 1nm to 100nm

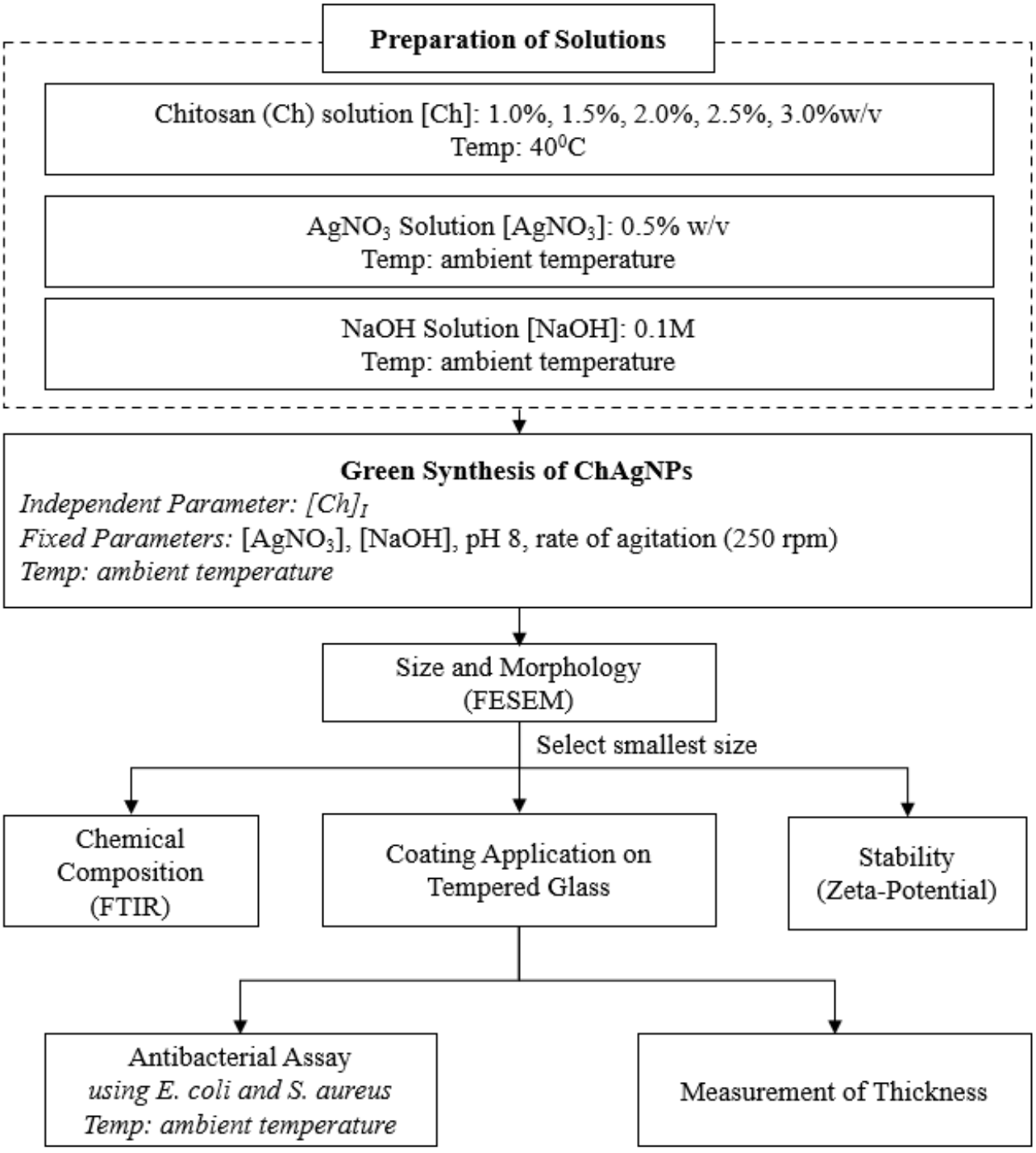

Figure 8. Methodological Framework

4.3. Green synthesis of ChAgNPs

The bottom-up approach in the green synthesis of ChAgNPs is the first procedure for the study. The succeeding subsections entail the preparation of the necessary solutions and the reduction reaction involve in the synthesis of the nanocomposite.

4.3.1. Sterilization of Apparatus, Glassware, Plastic Films, Screen Protectors, and Workplace

Prior to the proper antibacterial assay and coating procedures, aseptic techniques will be observed. This step is vital to all microbial studies to prevent contamination from unwanted pathogens. All the apparatus, glassware, and glass protectors will be sterilized in an autoclave at 121˚C for 15 minutes. On the other hand, the plastic films will be soaked in 70% isopropyl alcohol for 1 minute. After the experiment, the same sterilization procedures will be observed for all items. Furthermore, the working area during the duration of the experiments must be kept clean and free from cross-contamination. This can be done by the proper use of gloves in handling of the samples and by demarcation or separation of the working area from the rest of the laboratory. Surface sanitation with 70% isopropyl alcohol is done before and after the assay and the coating procedures and should also be done in case of accidental contamination during the experiments.

4.3.2. Preparation of chitosan solution

Glacial grade acetic acid (99.7% w/w) will be diluted to 2% w/v acetic with distilled water until 2 litres of solution is made. The diluted acetic acid will be used as solvent for chitosan powder with varying concentrations.

The concentration for chitosan solution to be considered are at 1.0, 1.5, 2.0, 2.5 and 3.0% w/v (g/mL). Chitosan concentration samples will be prepared by analytically weighing 2.50 g, 3.75 g, 5.00 g, 6.25 g, and 7.50 g of chitosan powder. This will then be diluted with the prepared acetic acid solution until 250 mL volume is reached in a volumetric flask. Agitation will be done for 1 hour at 40°C in an incubator shaker.

4.3.3. Reduction of Ag^+ ions to AgNPs

To prepare 0.5% w/v $AgNO_3$ solution, 1.25 g of $AgNO_3$ (≥ 99.0% purity) will be mixed with distilled water until 250mL of solution is achieved. Illustrated in Figure 9 is the reduction and capping synthesis of AgNPs using chitosan as a reagent. One hundred sixty millilitres (160 mL) of prepared chitosan solution will be added to 40mL of prepared $AgNO_3$ solution. Diluted NaOH solution (0.1M) will be added to the solution until pH reaches a reading of 8.0. Under magnetic stirring at around 250 rpm, the solution will be further reacted for 50 minutes on ambient temperatures. The prepared samples will then be transferred in an amber bottle to prevent the solution from photooxidation.

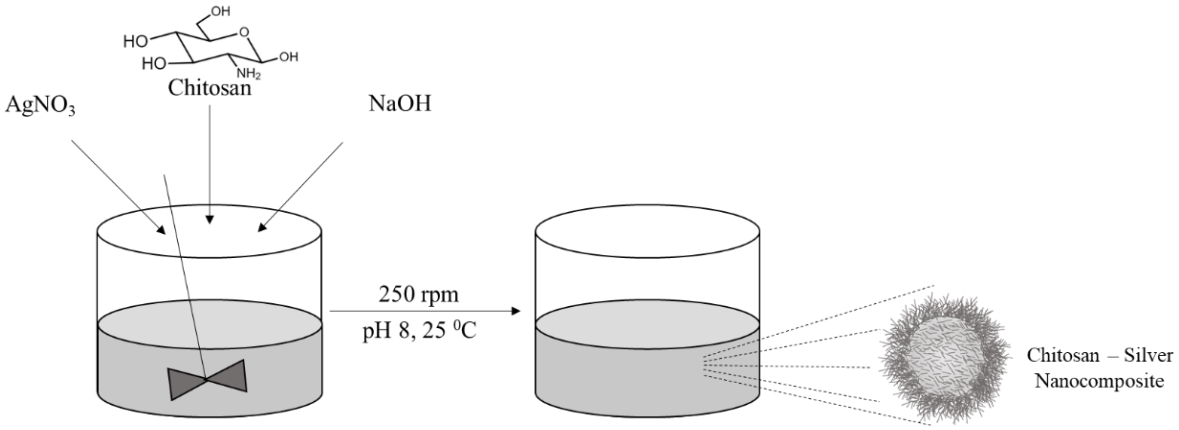

Figure 9. Illustration of the Molecular Perspective on the Reaction Synthesis of ChAgNPs from $AgNO_3$ Precursor

4.4. Characterization of ChAgNPs

The success of the formulation of miniscule materials such as nanocomposites will be identified through characterization methodologies and techniques. Furthermore, the methods examine the properties of the ChAgNPs under given conditions and observe its behavior. The characterization procedures are as follows:

4.4.1. Field Emission Scanning Electron Microscope (FESEM)

A. Size imaging

To measure the size of ChAgNPs at varying chitosan concentration, aliquots of each produced concentration sample are obtained from the synthesized solutions produced in Section 4.3. Using the Sigma-Zeiss 500 VP equipment, FESEM will be performed by air-drying each sample on a

carbon adhesive. The dried samples will be placed on an aluminium stub and will be coated with a thin layer of gold sputter through a rotary pump gold coater (Quorum Q150R S). The coated samples will be loaded to the apparatus and will be analysed at an accelerated voltage of 10kV.

The generated FESEM image of each sample will then subjected under image processing using ImageJ software for the determination of size measurement and distribution. A plot of average AgNPs size against chitosan concentration will be constructed along with its corresponding strandline and coefficient of correlation. The characterization procedures will be done at the University of San Carlos – Applied Physics Department because it has the FESEM equipment.

B. Selection of optimal ChAgNPs

Based on the results, a discrete selection among the produced ChAgNPs will be done. Based on the trend from size imaging, the obtained samples will be examined whether the average nanoparticle size falls within the definitive nanoparticle size range which is within 1 nm to 100 nm. Omitting the rejected samples, if any, the optimal ChAgNP and chitosan concentration will be selected based on the smallest average size from the analysed sample images. The optimal chitosan concentration shall be used for further characterization, coating application, and antibacterial assay.

4.4.2. Fourier Transform – Infrared (FT-IR) Spectroscopy

The purpose of the FT-IR is to confirm the composition of the nanocomposite in which both chitosan and AgNPs are present. This identifies a molecular “signature” which can indicate the presence of the substance within the sample system.

For the experiment process, the ChAgNPs nanocomposite at optimal chitosan concentration will be examined under a diffused reflective FT-IR (Perkin Elmer spectrum 100, USA) with attenuated total reflectance. Such equipment can conduct FT-IR analysis for both solid and liquid samples.

In this procedure, the ChAgNPs at optimal chitosan concentration from section 4.4.1.B is added with KBr pellets in a ratio (of KBr to sample) of 100:1. The sample will be studied such that the spectrum runs at 20 scans with each scan running at a range of 4000 – 600 cm^{-1} with a resolution of 0.09 cm^{-1}. The FTIR analysis shall be conducted at the University of San Carlos – Chemistry Department with available FTIR equipment.

4.4.3. Zeta Potential

Like FTIR analysis, the ChAgNPs at optimal chitosan concentration from section 4.4.1.B will be used for assessment of zeta potential. The sample will be transferred to an amber bottle and will be wrapped with bubble wraps to prevent the bottle from breaking. The bottle will then be placed in a box. Furthermore, the sample will be sent to the third-party institution via local carriers.

The optimal ChAgNPs is examined for zeta potential under a Horiba nanoPartica SZ-100-Z2 equipment operated with a voltage range of -200mV to +200mV. Sonicated sample is injected in a disposable carbon-coated cell with a volume of 100 μL in which the device measures the electrophoretic mobility of the particle surface. This will take place under at the Nanotechnology Research and Development (R&D) Facility of Central Luzon State University (CLSU) in Nueva Ecija, a third-party institution with available equipment in the Philippines.

4.5. Application of nanocomposite coating on tempered glass screen protector

In this study, the optimal ChAgNPs solution will be applied as a coating to tempered glass screen protectors. The procedures that will be done to attain the objective are detailed in the following subsections. Furthermore, this will be done using a Laminar Flow Hood for aseptic purposes.

4.5.1 Measurement of thickness of uncoated tempered glass

Twenty-four (24) commercial tempered glass screen protectors (2.5 cm x 2.5 cm) will be used for applications of the coating. The thickness of the tempered glass will be measured using the handheld micrometre. The procedure will follow the American Society for Testing and Materials (ASTM) Standards D1005: Procedure C for handheld micrometres.

To measure the thickness of the uncoated tempered glass, the anvil will be allowed to contact with the substrate, without compressing it, by rotating the adjustment knob. The coating thickness will then be recorded to 2.5 micrometres.

The thickness of the tempered glass will be read from the four corners of the panel and on the centre. Three different sets of thickness measurement will be done for each substrate.

4.5.2. Drop-casting method

This section covers the coating procedure that will be implemented in this study. A modified method of Bormashenko et al. (2019) and Chandren & Zulfemi (2019) will be used in the drop-casting procedure of this study.

The amber glass bottle with the solution containing the optimal chitosan-silver nanocomposites will be prepared. The tempered glass substrates will then be placed on a flat surface. The prepared solution, as described in Section 4.3.2 will be casted on the sterilized tempered glass substrates – see Section 4.3.1. for the sterilization process – by dropping on the surface. A 1-mL micropipette containing 0.2 mL of the solution will be positioned 15 cm above the glass substrate. Then, the four corners and the centre of the glass substrate will be dropped with 0.05 mL of the chitosan-silver nanocomposite.

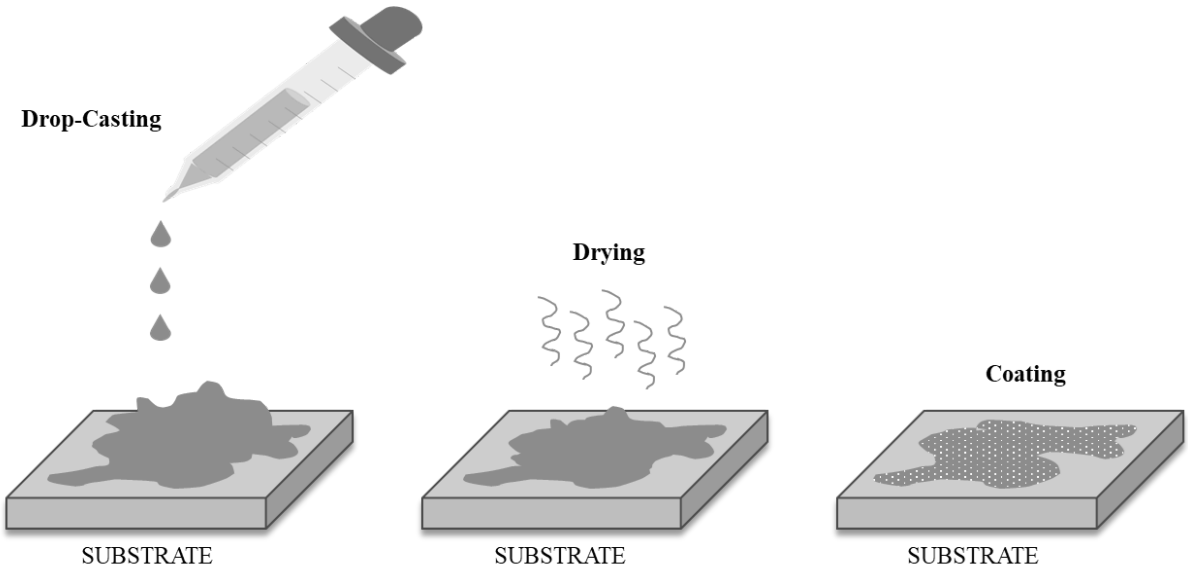

Figure 10. Visual Representation of Drop-Casting Method (Adapted from Bormashenko et al., 2019)

The drop-casting method is illustrated in Figure 10. The drop-casting process will be done under room temperature. The substrate will be left to dry at room temperature. After the substrate has dried, it will be carried for curing process.

4.5.3 Curing

To ensure the adhesion of the ChAgNP coating onto the glass surface, a physical curing method was adapted from Vieira et al. (2014). The coated tempered glass will be oven dried with at 50.8˚C for 12 hours.

4.5.4 Measurement of thickness of coating

The same procedure (*see section 4.5.1*) will be used in the measurement of the coated tempered glass.

The coated screen protector will be placed perpendicularly between the anvil contacts. To measure the thickness of the coating, the anvil will be allowed to contact with the substrate, without compressing it, by rotating the adjustment knob. The coating thickness will then be recorded to 2.5 micrometres. A reading will then be recorded estimating to 2.5 micrometres.

The thickness of the coating will be read from the four corners of the panel and on the centre. Three different sets of thickness measurement will be done for each substrate. The difference in the thickness measurements of the coated and uncoated tempered glass is the thickness of the coating.

4.6 Antibacterial assay for ChAgNP coating

The antibacterial assay is meant to assess the antibacterial activity of the ChAgNP coating by determining the log reduction of the number of bacteria on the coated surface after a specific contact time (i.e., duration of time the glass is in contact with the bacteria). The ISO 22196 Test for Antibacterial Activity of Plastic Surfaces and Other Non-Porous Surfaces will be adapted. This method is designed to quantitatively test the ability of a plastic or non-porous surface to inhibit the growth or to kill them. It is usually done over a 24-hour contact time but could be modified to shorter periods according to study objectives. Currently, this is the most popular test protocol for testing the antibacterial activity of a surface (Campos et al., 2016).

The optimal ChAgNP coating (with smallest particle size) will be tested for antibacterial activity at different contact times (1 min, 1 hr, 2 hrs, and 24 hrs). The bacteria to be tested will be the gram-positive *S. aureus* and the gram-negative *E. coli*. The positive infection control will be represented by uncoated glass surfaces. Samples and control will be tested in triplicates. The assay will be performed by and at the University of San Carlos Biology Department.

The ChAgNP coating solution will be transferred to an amber bottle and packaged with bubble wrap to prevent breakage. Together with the glass protectors, the solution-containing amber bottle will be placed in a black box. These will be sent to the third-party institution via local carriers.

The following antibacterial assay procedure was adapted from ISO 22196 (International Organization for Standardization: Measurement of antibacterial activity on plastics and other non-porous surfaces) and the modified version of this by Campos et al. (2016) who investigated film screen protectors instead of glass.

4.6.1. Preparation of Growth Medium

To prepare the broth, 21 g of Mueller-Hinton broth powder will be dissolved in 1L of distilled water. Ensure that the solution is mixed well. It will be sterilized by autoclaving at 121˚C for 15 minutes.

To prepare the agar, 38 g of Mueller-Hinton broth powder will be dissolved in 1L of distilled water. Ensure that the solution is mixed well. It will be sterilized by autoclaving at 121˚C for 15 minutes. The resulting liquid will be poured into petri dish and solidified.

4.6.2. Preparation of Bacterial Culture

Ten mL of Mueller-Hinton broth (MHB) will be incubated overnight in a shaker at 37˚C. On the day of testing, 200 µL of the bacterial culture will be grown in 10 mL of fresh MHB and will then be subject to shaking at 37˚C for 1 hour to reach the exponential growth phase (Campos et al., 2016). The resulting culture will be further diluted in 0.9% (w/v) sodium chloride solution to a target cell density of 7 $\log_{10}$ colony forming units (CFU)/mL.

4.6.3 Application of bacteria onto surface

The glass surfaces will be transferred each to a Petri dish with the test surface uppermost. Each glass surface will be inoculated with 50 µL of the prepared culture from Section 4.6.2 and then will be covered and spread with a sterilized plastic film measuring 20 mm x 20 mm as per ISO 22196. It should be ensured that the test inoculum does not leak beyond the edges of the film. This is to prevent the sample from drying and to ensure maximum contact. The samples will then be incubated at 25˚C or room temperature in a black box which will also contain pieces of paper saturated with water to maintain high humidity.

4.6.4 Recovery of bacterial inoculum

After a certain contact time (1 min, 1 hr, 2 hrs, and 24 hrs) at 25˚C or room temperature, the glass surfaces and the films will be submerged in 20 mL of a 0.9% (w/v) sodium chloride solution in a 100 mL beaker. The beaker will be covered with aluminium foil and will be agitated

with a sonicator for 30 seconds. One mL of the resulting solution will be serially diluted (10-fold until 10^{-10}), then plated on Mueller-Hinton agar (MHA), and then incubated for 24 h at 37˚C.

4.6.5 Determination of antibacterial activity

After incubation, the CFU per plate (U_t and A_t) will be determined. However, only the plate counts of 30-300 colonies will be considered valid. The antibacterial activity (in %) after a certain contact time will be calculated using Equation 1.

4.7 Statistical analysis

The design of experiment is an essential tool to allow analysis and validate the results and conclusion based on the desired objectives. Thus, for every experiment, a systematic approach employing the study on how the factors affect the results of the experiment. In this experiment, the optimal chitosan-silver nanocomposite solution will be tested for its antibacterial activity as a glass screen protector coating against *S. aureus* and *E. coli* under four different contact times. On the same antibacterial assay, the positive infection control will be the uncoated glass screen protector. Hence, the study will involve two separate groups (coated and uncoated) with each group, evaluated four different contact times. This experimental setup will be applied for both bacteria. Therefore, in this study the obtained data will be processed using analysis of variance (ANOVA).

4.7.1 Determination of antibacterial activity using the R-value

The antibacterial activity will be measured in terms of the log reduction of the number of bacteria (in colony-forming units or CFU) on the test sample (in colony-forming units) relative to that of the positive infection control (uncoated surface). This calculation for the antibacterial activity R_t is represented by Equation 1 as adapted from ISO 22196. The CFU values to be used in the computations will be the mean average of only the valid plate counts (30-300 colonies per plate). There will be four different contact times at which the antibacterial activity will be measured.

$$R_t = \left(U_t - A_t\right) \times 100\% \quad (1)$$

Such that,

U_t = mean common logarithm of CFU of positive infection control after a time

A_t = mean common logarithm of CFU of sample after a time

4.7.2 Analysis of Variance

In the case of having three or more data sets, the analysis of variance (ANOVA) could be employed. Like the *t*-test, the ANOVA is designed to determine whether there is a statistical difference between the differences between the means of the group. However, this statistical analysis should only be employed if the data are normally distributed and that the variances are homogenous. In an ANOVA the following hypotheses are used:

$$H_0 = \mu_1 = \mu_2 = \ldots = \mu_n \quad (2)$$

$$H_1 = at\ least\ one\ \mu_i\ is\ different \quad (3)$$

Where H_0 ,H_1, and k denote the null hypothesis, alternative hypothesis, and the number of groups respectively. The null hypothesis will be rejected when there is at least one mean (μ_i) that is significantly different from the means from the group (Bluman, 2008).

The only parameter that was varied during the antibacterial assay on each bacterium was the contact time. Hence, a one-factor ANOVA can be employed. The test statistic for an ANOVA is the *F-statistic*, mathematically expressed in Equation 4.

$$F = \frac{variance\ among\ treatments}{variance\ within\ treatments} \quad (4)$$

In the case that there is no significant difference between the two variances ($F \approx 1$), the null hypothesis will not be rejected. However, if there is a difference among the means ($F \gg 1$), then the null hypothesis will be rejected (Levine et al., 2009).

V. References

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VI. Cost Estimates

6.1 Proposed Budget for Materials

Item Description	*Quantity*	*Cost/Unit*	*Subtotal*	
			Based on base quantity	*Based on estimated usage*
[1] Chitosan Powder	50 g	149.45/g	37,362/250g	7,472.40
[2] Glacial Acetic Acid	40 mL	0.80/mL	2,000/2.5L	32.00
[3] Silver Nitrate	5 g	100/g	10,000/100g	500.00
[4] Sodium Hydroxide	8 g	1.10/g	1,100.00/kg	8.80
[5] Distilled Water	3 gal	100.00/gallon	100.00/gallon	300.00
[6] *S. aureus* Culture	1	500/culture	500/culture	500.00
[7] *E. coli* Culture	1	500/culture	500/culture	500.00
[8] Mueller Hinton Agar/Broth	60 g	20/gram	20/gram	1,200.00
[9] 0.9% Saline Solution	1 L	120/L	120/L	120.00
[10] Plastic Film	1 m	2.25/m	45/20m	2.25
[11] Aluminium Foil	20 sheets (12”x10.75”)	3.5/sheet	140/40 sheets	70.00
[12] Lint-free Tissue	2 boxes	80.00/box	80.00/box	160.00
[13] Tempered Glass	47 pcs	59.00/pc	59/pc	2,832.00
[14] 500mL Amber bottles	7 pcs	54.00/bottle	54.00/bottle	378.00
[15] 70% Isopropyl Alcohol	1 L	200/L	100/500mL	200.00
[16] General Lab Supplies	---	---	---	8,000.00
		Total		**22,275.45**

6.2 Proposed Budget for Laboratory Equipment/Apparatus Use

Item Description	*Number of Hour/ samples*	*Cost/hour/sample*	*Subtotal*
[1] Field Emission Scanning Electron Microscope	5 samples	3,000/sample	15,000.00
[2] Fourier Transfer Infrared Spectroscopy	1 hr	1,500/hr	1,500.00
[3] Horiba nanoPartica Equipment	1 hr	2,000/hr	2,000.00
[3] Magnetic Stirrer	8 hrs	40/hr	320.00
[4] Digital pH meter	7 hr	72/hr	504.00
[5] Thermometer	7 hrs	10/hr	70.00
[6] Incubator shaker	2 hrs	40/hr	80.00
[7] Sonicator	1 hr	300/hr	300.00
[8] Incubator	48	600/24 hrs	1,200.00
[9] Dry Oven	48 hr	600/ 24 hrs	1,200.00

[10] Analytical Balance	5 uses	220/use	1,100.00
[11] Laminar Flow Hood	8 hrs	200/hr	1.600.00
[12] Other Laboratory Apparatus	----	-----	5,000.00
		Total	**29,794.00**

6.3 Room Rental Costs

Room	*No. of hours*	*Cost/hour*	*Subtotal*
[1] ChE Research Laboratory	24	250/hr	6,000.00
		Total	**6,000.00**

6.4 Miscellaneous Costs

External Analysis			
Description	*No. of Days*	*Analysis Fee*	*Subtotal*
[1] Antibacterial Assay	4	2,000.00/day	8,000.00
		Total	8,000.00
Documentation Costs			
Description	*Quantity*	*Cost/Unit*	*Subtotal*
[1] Internet Connection	10 months	250.00/month	2,500.00
[2] Bond paper	2 reams	500.00/pc	1,000.00
[3] Printer Ink	10 pcs	250.00/pc	2,500.00
[4] Poster	1 pc	500.00/pc	500.00
[5] Binding	2 pcs	200.00/pc	400.00
		Total	6,900.00
Transportation and Accommodation Costs			
Description	*No. of samples*	*Rate/sample*	*Subtotal*
[1] ChAgNPs Shipment to CLSU	1	500.00/sample	500.00
		Total	500.00
Incidental Costs			
Description	*Quantity*	*Cost/Unit*	*Subtotal*
[1] Chemical Waste Disposal	3L	300.00/L	900.00
[2] Waste Container	2 containers	75.00/container	150.00
		Total	1,050.00
		Overall Total	**16,450.00**

6.5 Summary of Costs

Item Description	*Subtotal*
[1] Materials	22,275.45
[2] Laboratory Equipment and Apparatus Use	29,794.00
[3] Room Rental Costs	6,000.00
[4] Incidental Costs	16,450.00
Total	**74,519.45**

[5] *Contingency cost (20% of the total cost)*	14,903.89
Grand Total	***89,423.34***

VII. Workplan and Expected Output

Activity/Task	*Aug*							*Expected Output*	*Person(s) Responsible*
	16	17	18	19	20	21	22		
[1] Preparation of Form 1 (Notice of Acceptance)				■				Filled-up Notice of Acceptance	Canama Delco Talandron
[2] Meet with the adviser for research group orientation						■		Information regarding the chosen topic	Canama Delco Talandron Dr. Tan
[3] Send the Notice of Acceptance to the Adviser						■		Sent email to adviser for signature	Canama
Activity/Task	*Aug*							*Expected Output*	*Person(s) Responsible*
	23	24	25	26	27	28	29		
[4] Submit Notice of Acceptance	■							Successfully submitted Notice of Acceptance to the coordinator	Canama
[5] Research about Chitosan		■	■					Information compilation about the assigned topic	Talandron
[6] Research about the antibacterial activity of Chitosan		■	■					Information compilation about the assigned topic	Delco
[7] Research about antibacterial coating applications of Chitosan		■	■					Information compilation about the assigned topic	Canama
[8] Consolidate and discuss information with the group				■				Compiled consolidated information	Canama Delco Talandron
Activity/Task	*Aug/Sep*							*Expected Output*	*Person(s) Responsible*
	30	31	1	2	3	4	5		
[9] Formulate initial research idea from the consolidated information				■				Initial Research Idea	Canama Delco Talandron
[10] Create a power point presentation about the proposed research topic				■				Power point presentation	Canama Delco Talandron
[11] Propose the research topic to the adviser					■			Inputs from the adviser and suggested edits	Canama Delco Talandron Dr. Tan
[12] Prepare activity log					■			Filled activity log	Canama
[13] Send the activity log to the adviser					■			Successfully signed activity log	Canama
[14] Apply suggested edits to the research topic					■	■	■	Edited research proposal	Canama Delco Talandron

Activity/Task	*Sep*							*Expected Output*	*Person(s) Responsible*
	6	7	8	9	10	11	12		
[15] Propose the new research topic to adviser								Approved research proposal topic	Canama Delco Talandron Dr. Tan
[16] Consult with the adviser on how to go about with the research topic								Minutes of the meeting	Canama Delco Talandron Dr. Tan
[17] Establish an Initial Research Title								Initial Research Title	Canama Delco Talandron Dr. Tan
[18] Prepare activity log								Filled activity log	Delco
[19] Send the activity log to the adviser								Successfully signed activity log	Canama
[20] Division of Tasks for proposal manuscript								Task Distribution	Talandron
[21] Research about bacterial nosocomial infection								Information about nosocomial infection	Talandron
[22] Research on the contribution of mobile phones on nosocomial bacterial infection								Information about the contribution of mobile phones on nosocomial infection	Delco
[23] Research on *S. aureus* and *K. pneumoniae*								Information on the bacteria	Delco
[24] Research on current technologies								Literature on current technologies	Canama
[25] Research about silver nanoparticles								Literature on silver nanoparticles	Canama Delco Talandron
[26] Research on the antibacterial activity of silver nanoparticle								Literature on the antibacterial activity of silver nanoparticles	Delco
[26] Research on the antibacterial activity of chitosan								Literature on the antibacterial activity of silver nanoparticles	Talandron
[27] Research on the antibacterial activity of chitosan-silver nanocomposites								Literature on the antibacterial activity of chitosan-silver nanocomposites	Talandron Delco
[28] Research on characterization of nanoparticles								Literature on the characterization of nanoparticles	Canama
[29] Consolidate and discuss all gathered information								Compiled information and manuscript inputs	Canama Delco Talandron

[30] Generate mind map					■			Mind map	Canama Delco Talandron
[31] Generate initial bibliography					■			Initial Bibliography	Canama Delco Talandron
[32] Submit mind map and initial bibliography to adviser for checking					■			Approval from adviser	Canama Dr. Tan
[33] Submit mind map and initial bibliography to coordinator						■		Successfully submitted mind map and initial bibliography to coordinator	Canama
Activity/Task	*Sep*							*Expected Output*	*Person(s) Responsible*
	13	14	15	16	17	18	19		
[34] Research on the Green Synthesis methods of chitosan-silver nanocomposite and coating method	■	■	■					Excel sheet containing the information of the synthesis methods	Canama Delco Talandron
[35] Research on common polymers used in the synthesis of AgNPs	■	■	■					Excel sheet literature on the common polymers used in the AgNPs	Talandron
[36] Consolidate and discuss the gathered information			■					Consolidated information	Canama Delco Talandron
[37] Input information in the manuscript			■	■				Subsection of green synthesis in the manuscript; Illustration of synthesis method	Canama Delco Talandron
[38] Consultation with adviser					■			Minutes of the meeting, Corrected synthesis and coating method	Canama Delco Talandron Dr. Tan
[39] Application of suggestions and corrections					■			Synthesis method with applied correction	Canama Delco Talandron
[40] Research on the factor affecting the synthesis of the nanocomposite						■	■	Information about the factors affecting the synthesis process	Canama Delco Talandron
Activity/Task	*Sep*							*Expected Output*	*Person(s) Responsible*
	20	21	22	23	24	25	26		
[40] Research on the factor affecting the synthesis of the nanocomposite	■	■	■	■	■	■		Information about the factors affecting the synthesis process	Canama Delco Talandron
[41] Consolidate and discuss the factor affecting the synthesis process						■	■	Consolidated and agreed information	Canama Delco Talandron
Activity/Task	*Sep/Oct*							*Expected Output*	*Person(s) Responsible*
	27	28	29	30	1	2	3		

[42] Consolidate and discuss the factors affecting the synthesis process	■	■	■					Consolidated and agreed information	Canama Delco Talandron
[43] Place the agreed factors in the manuscript			■		■			Manuscript sub section for factors affecting the synthesis process	Canama Delco Talandron
Activity/Task	*Oct*							*Expected Output*	*Person(s) Responsible*
	4	5	6	7	8	9	10		
[44] Research on the role of Chitosan on the synthesis process	■	■	■	■				Information regarding the role of chitosan	Canama Delco Talandron
[45] Submit initial write-up for theoretical background to adviser	■							Corrected manuscript (theoretical background)	Canama Dr. Tan
[46] Consolidate and discuss the gathered information					■			Agreed roles of chitosan	Canama Delco Talandron
[47] Input the statement for the role of chitosan in the manuscript						■	■	Manuscript subsection for the role of chitosan	Canama Delco Talandron
Activity/Task	*Oct*							*Expected Output*	*Person(s) Responsible*
	11	12	13	14	15	16	17		
[48] Consultation with Adviser	■							Minutes of the Meeting Corrected Factors of Synthesis	Canama Delco Talandron Dr. Tan
[49] Application of suggestions and corrections		■	■	■					Canama Delco Talandron
[50] Continue the write-up for theoretical background (application of edits suggested by adviser)					■	■	■	Write-up for theoretical background	Canama Delco Talandron
Activity/Task	*Oct*							*Expected Output*	*Person(s) Responsible*
	18	19	20	21	22	23	24		
[51] Continue the write-up for theoretical background (application of edits suggested by adviser)	■	■	■	■				Write-up for theoretical background	Canama Delco Talandron
[52] Establish the significance, scopes, and limitations of the study				■	■			Scopes and Limitations Significance of the study	Canama Delco Talandron
[53] Consultation with adviser						■		Minutes of the meeting Corrected scopes and Limitations, and significance of the study	Canama Delco Talandron Dr. Tan
[54] Send manuscript to adviser (scopes and limitations, significance, methods)						■		Annotated manuscript	Canama

Activity/Task	*Oct*							*Expected Output*	*Person(s) Responsible*
	25	26	27	28	29	30	31		
[55] Revision of manuscript	■	■	■	■	■	■	■	Revised manuscript	
[56] Finalized list of chemicals	■							Final list and amounts of chemicals	Canama Delco Talandron
[57] Contact the ChE Laboratory	■							Price quotations	Canama
Activity/Task	*Nov*							*Expected Output*	*Person(s) Responsible*
	1	2	3	4	5	6	7		
[56] Revision of manuscript	■	■						Revised manuscript	Canama Delco Talandron
[57] Communicate with laboratories			■	■	■			Quotations and protocols Formal Letters Email Communication	Canama
[58] Finalize manuscript				■	■			Finalized manuscript	Canama Delco Talandron
[59] Send to the manuscript to the adviser for approval and endorsement					■			Approved proposal manuscript	Canama
[60] Submit manuscript to the coordinator						■		Submitted proposal manuscript	Canama
Activity/Task	*Nov*							*Expected Output*	*Person(s) Responsible*
	8	9	10	11	12	13	14		
[61] Making of power point presentation	■	■	■	■	■	■	■	Initial Power point presentation	Canama Delco Talandron
[62] Submission of power point to adviser for checking							■	Corrected power point presentation	Canama Delco Talandron Dr. Tan
Activity/Task	*Nov*							*Expected Output*	*Person(s) Responsible*
	15	16	17	18	19	20	21		
[63] Finalization and practice for proposal hearing	■	■	■	■	■	■	■	Final Power point presentation	Canama Delco Talandron Dr. Tan
Activity/Task	*Nov*							*Expected Output*	*Person(s) Responsible*
	22	23	24	25	26	27	28		
[67] Proposal hearings	■	■	■	■	■	■	■	Approval from panel, Panel suggestions, Minutes of discussion	Canama Delco Talandron Dr. Tan
Activity/Task	*Nov/Dec*							*Expected Output*	*Person(s) Responsible*
	29	30	1	2	3	4	5		

[68] Revision of Manuscript								Revised Proposal	Canama Delco Talandron Dr. Tan
Activity/Task	*Dec*							*Expected Output*	*Person(s) Responsible*
	6	7	8	9	10	11	12		
[69] Submission of Manuscript with endorsement from adviser								Final Research Manuscript	Canama Delco Talandron Dr. Tan
Activity/Task	*Dec*							*Expected Output*	*Person(s) Responsible*
	13	14	15	16	17	18	19		
[70] Preparation for experiments								Finalized agreements and schedules with concerned laboratories Finalized inventory for chemicals, apparatus, and equipment	Canama Delco Talandron
Activity/Task	*Jan*							*Expected Output*	*Person(s) Responsible*
	17	18	19	20	21	22	23		
[71] Review of experimental procedures									Canama Delco Talandron
[72] Synthesis of Chitosan-Silver Nanocomposite								Chitosan-Silver Nanocomposite solutions	Canama Delco Talandron
Activity/Task	*Jan*							*Expected Output*	*Person(s) Responsible*
	24	25	26	27	28	29	30		
[73] Characterization: FESEM								FESEM Image Optimum ChAgNPs nanocomposite concentration	Canama Delco Talandron
[74] Characterization: FTIR								FTIR Data	Canama Delco Talandron
[75] Characterization: Zeta-potential								Zeta-potential data	Canama Delco Talandron
Activity/Task	*Jan/Feb*							*Expected Output*	*Person(s) Responsible*
	31	1	2	3	4	5	6		
[76] Characterization: Zeta-potential								Zeta-potential Data	Canama Delco Talandron

[77] Application of Coating								Coated tempered glass	Canama Delco Talandron
[78] Antibacterial assay								Antibacterial assay data	Canama Delco Talandron
Activity/Task	*Feb*							*Expected Output*	*Person(s) Responsible*
	7	8	9	10	11	12	13		
[79] Data Processing and interpretation for FTIR spectrum results								Processed Data and Inferences for FTIR	Canama Delco Talandron
[80] Data Processing and interpretation Stability results								Processed Data and Inferences for Stability	
Activity/Task	*Feb*							*Expected Output*	*Person(s) Responsible*
	14	15	16	17	18	19	20		
[81] Data Processing for Antibacterial Assay								Processed Data and Inferences for Antibacterial Assay	Canama Delco Talandron
Activity/Task	*Feb*							*Expected Output*	*Person(s) Responsible*
	21	22	23	24	25	26	27		
[82] Writing of research manuscript (results and discussion)								Initial research manuscript (results and discussion)	Canama Delco Talandron
Activity/Task	*Feb/Mar*							*Expected Output*	*Person(s) Responsible*
	28	1	2	3	4	5	6		
[83] Writing of research manuscript (results and discussion)								Initial research manuscript (results and discussion)	Canama Delco Talandron
Activity/Task	*Mar*							*Expected Output*	*Person(s) Responsible*
	7	8	9	10	11	12	13		
[84] Writing of research manuscript (results and discussion)								Initial research manuscript (results and discussion)	Canama Delco Talandron
Activity/Task	*Mar*							*Expected Output*	*Person(s) Responsible*
	14	15	16	17	18	19	20		
[85] Send to adviser for revisions								Corrected manuscript	Canama Delco Talandron Dr. Tan

Activity/Task	*Mar*							*Expected Output*	*Person(s) Responsible*
	21	22	23	24	25	26	27		
[86] Revise the manuscript								Revised manuscript	Canama Delco Talandron
Activity/Task	*Mar/Apr*							*Expected Output*	*Person(s) Responsible*
	28	29	30	31	1	2	3		
[87] Submission of Revised Manuscript to Adviser								Comments on revision of manuscript	Canama Delco Talandron Dr. Tan
Activity/Task	*Apr*							*Expected Output*	*Person(s) Responsible*
	4	5	6	7	8	9	10		
[88] Revise the manuscript								Revised manuscript	Canama Delco Talandron
Activity/Task	*Apr*							*Expected Output*	*Person(s) Responsible*
	11	12	13	14	15	16	17		
[89] Finalize the manuscript								Finalized manuscript	Canama Delco Talandron Dr. Tan
Activity/Task	*Apr*							*Expected Output*	*Person(s) Responsible*
	18	19	20	21	22	23	24		
[90] Submission of Manuscript								Submitted manuscript	Canama Delco Talandron Dr. Tan
[91] Making of power point presentation								Power point presentation	Canama Delco Talandron
Activity/Task	*Apr/May*							*Expected Output*	*Person(s) Responsible*
	25	26	27	28	29	30	1		
[92] Finalization and practice								Power point presentation	Canama Delco Talandron Dr. Tan
Activity/Task	*May*							*Expected Output*	*Person(s) Responsible*
	2	3	4	5	6	7	8		

[93] Oral defence								Approval from Panel Corrected manuscript; Recommendation from panel, Minutes of Discussion	Canama Delco Talandron
Activity/Task	*May*							*Expected Output*	*Person(s) Responsible*
	9	10	11	12	13	14	15		
[94] Revise Manuscript								Revised manuscript	Canama Delco Talandron
Activity/Task	*May*							*Expected Output*	*Person(s) Responsible*
	16	17	18	19	20	21	22		
[95] Finalized Manuscript								Finalized Manuscript	Canama Delco Talandron Dr. Tan
[96] Submission of Final Manuscript								Submitted final research manuscript	Canama Delco Talandron

VIII. Appendices

8.1. Preparation of Solutions

In the synthesis of ChAgNPs, acetic acid and sodium hydroxide solutions are required. The following subsections details the preparation of the above-mentioned solutions.

8.1.1. Preparation of Acetic Acid Solution

The preparation of 2% w/v Acetic Acid solution will be done by mixing 38.2 mL of concentrated glacial grade acetic acid (99% purity) with 1L of distilled water with thorough mixing. Further dilution was made by adding more distilled water until 2L of solution is achieved.

8.1.2. Preparation of Sodium Hydroxide Solution

To prepare the 350 mL of 0.1M NaOH solution, 1.399 g of NaOH pellets will be weighed. The NaOH pellets will then be dissolved in 350 mL of distilled water. Afterwards, the NaOH solution will be stored in a labelled container.

8.2. Calibration of pH Meter

Prior to the calibration procedure, the pH meter will be turned on and will be allowed to warm up for 5 minutes. The pH meter probes will then be rinsed with distilled water and will be wiped with lint-free tissue to dry. Afterwards, two buffers for calibrating the pH meter will be made. One with a pH of 7 for the neutral buffer, and a pH of 4 for the acidic buffer. These buffers will be placed in beakers for calibration. The probe will be placed in the appropriate buffer and measure the pH of the calibration buffer. The pH meter will be allowed to stabilize the reading and afterwards, the pH meter will be set to the value of buffer's pH. After the calibration procedure, the probes will be rinsed with distilled water and dried using a lint-free tissue.