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The effect of glycerin and polyethylene glycol 400 as humectant on stability and antibacterial activity of nanosilver biosynthetic peel-off mask

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ABSTRACT

The peel of sweet orange contains flavonoids and acts as a bioreducer to silver ion, thereby forming nanosilver (AgNPs) that act as an active ingredient in a peel-off mask. Meanwhile, glycerin and polyethylene glycol 400 (PEG 400) are humectants that are possibly combined to control viscosity and keep the skin moist. Therefore, this study aims to determine the effect of combined humectant on the stability and antibacterial activity of the AgNPs peel-off mask. The biosynthesis process was performed by mixing sweet orange peel infusion and silver nitrate solution, after which it was characterized using UV-Vis spectrophotometer, particle size analysis, and Scanning Electron Microscope. Five formulas were made with varying concentrations of humectant during the stability tests of the peel-off mask, and they include organoleptic, viscosity, pH, dry time, and antibacterial activity. Sweet orange peel infusion produces nanosilver with absorption at 440 nm and a particle size of 59.3–64.6 nm, being rod-shaped, and having a strong antibacterial activity. The result of the statistical analysis shows that glycerin-PEG 400 affects viscosity, dry time, and pH. Additionally, a combination of 0.375 and 0.125 g glycerin-PEG 400 showed the best physicochemical stability and had a moderate antibacterial activity after the cycling test compared to another formula.

INTRODUCTION

The silver metal was used as an antiseptic and a broad spectrum of biocidal activity (Ahmed *et al.*, 2016). Furthermore, it is produced in nanosize to increase antibacterial activity and stability when used as an active ingredient in cosmetic preparation (Sakharwade, 2016). Nanosilver penetrates the bacterial cell wall and changes the structure and permeability of the cell membrane, thereby killing the bacteria (Prabhu and Poulose, 2012). The minimum effective concentration of nanosilver in cosmetic preparations is equivalent to 10 mg/kg w/w (Pulit-Prociak *et al.*, 2019). Meanwhile, nanosilver with a size of 20–200 nm only penetrates the stratum corneum as far as 2–3 µm (stratum corneum is the outermost layer of the skin with a thickness of 15–20 µm), thereby preventing it from penetrating the systemic tract (Campbell *et al.*, 2012). However, the Science Committee on Consumer Safety limits its concentration in the body not to exceed 10,000 ppm (Pulit-Prociak *et al.*, 2019). Nanosilver in cosmetic preparations was designed to use topical antibiotics such as Clindamycin and Erythromycin to help cure skin problems, namely, acne (Fox *et al.*, 2016). Therefore, long-term use of topical antibiotics is not recommended because it causes bacterial resistance (Coates *et al.*, 2002).

The green synthesis method for the formation of nanosilver has advantages over physical and chemical because it is simple, cost-effective, environmentally friendly, and easier to upgrade on a larger scale (Dhupwer *et al.*, 2012). The biosynthesis process of nanosilver requires a reducing and capping agent. Reductants reduce the particle size as well as stabilizing the size of nanosilver (Ahmed *et al.*, 2016). Hydroxyl and carbonyl groups in plant extract are known as reducing and capping agents (Hembram *et al.*, 2018). Sweet orange functions as a bioreducer (*Citrus sinensis* L. Osbeck), as shown by the previous research in reducing silver nitrate ($AgNO_3$) by producing a particle size of 10–35 nm (Ahmed *et al.*, 2016) and smaller size of nanosilver compared with the same genus as lemon (*Citrus limon*) and sweet lime (*Citrus limetta*). Furthermore, the biosynthesis process with *C. limon* produces nanosilver with a size of 17.3–61.2 nm, while

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C. limetta produces nanosilver with a size of 107 nm (Dutta *et al.*, 2020; Nisha *et al.*, 2014). Orange peel contains a flavonoid of 5.51 mg/g and citric acid of 53.67 mg/g (Canan *et al.*, 2016; Liew *et al.*, 2018), which have hydroxyl and carbonyl groups that are capable of forming nanosized particles of silver (Malassis *et al.*, 2016). The results showed that the nanosilver using bioreducer of sweet orange peel infusion at 60°C has an inhibitory diameter against *Escherichia coli* of 16 mm, *Pseudomonas aeruginosa* of 13.4 mm, and *Staphylococcus aureus* 9.2 mm. Additionally, Logeswari *et al.* (2012) reported that 100 µl of nanosilver has a diameter of inhibition against *S. aureus* of 27 mm, *P. aeruginosa* of 18 mm, *E. coli* of 17 mm, and *Klebsiella pneumoniae* of 16 mm. Studies also showed that nanosilver has antibacterial activity against *S. aureus* with a diameter of inhibition of 28 mm, *E. coli* of 30 mm, *Bacillus cereus* 25 mm, and *Salmonella typhimurium* of 30 mm.

A peel-off mask is advantageous because it is easy to apply, leaves no residue when removed, and gives a clean sensation. Furthermore, its preparation optimizes the nanosilver as an antimicrobial agent when the polymer forms an occlusive layer on the stratum corneum skin surface (Velasco *et al.*, 2014). Humectants in peel-off mask preparations have two functions, which include hydrating the skin by pulling water from the inner layer to the outermost layer and preventing water evaporation for a more stable viscosity (Baki and Alexander, 2015). They also complement the function of polyvinyl alcohol (PVA) to form a soft and sturdy film in humid conditions (Ogur, 2005). Polyethylene glycol 400 (PEG 400) and glycerin are humectants and function as moisturizers with a concentration of 0.01%–20% (Liu, 2018), while PEG 400 is a material that maintains the viscosity of cosmetics. Furthermore, glycerin prevents skin irritation and maintains skin moisture in the long term (Benson *et al.*, 2019). This study aims to determine the effect of the combined humectants on the physicochemical character, stability, and antibacterial activity. The expected result is to obtain the formula of nanosilver peel-off mask that meets the requirements of quality, stability, and broad-spectrum antibacterial activity with a strong category.

MATERIALS AND METHODS

Materials and instruments

The materials used are sweet orange fruits from Pacitan, Central Java, Indonesia, silver nitrate powder ($AgNO_3$) 99.8% (Merck, Darmstadt, Germany), PVA (Kurray Asia Pacific PTE LTD, Singapore), PEG 400 (PT. DOW Chemical, Indonesia), glycerin (P&G Chemical, Singapore), aquabidest as a solvent, phenoxyethanol (MakingCosmetics, USA), Mueller Hinton agar (MHA) media (Merck, German), *Staphylococcus epidermidis* ATCC 12228, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922.

Meanwhile, the instruments used are pH meter (PH-009(I)A, China), analytical balance (Mettler Toledo AL204, d = 0.0001 g Colombus, Ohio), analytical balance (Precisa XB620C, d = 0.01 g Moosmattstrasse, Swiss), spectrophotometer UV-Vis (GenesysTM, Thermo Fisher Scientific, USA), centrifugation (Mini Spin Plus, Eppendorf AG, Jerman), viscometer (Viskotester VT-04, Kokubunji, Jepang), incubator (Memmert IN30, Mammert, Jerman), PSA is Particle Size Analyzer (HORIBA, USA), and SEM is Scanning Electron Microscope , ATCC is The American Type Culture Collection.

Biosynthesis process of silver ion using sweet orange infusion as bioreductor

Sweet orange plant harvested from Jetis Lor Village, Pacitan, Central Java, Indonesia, was determined in the Biology Laboratory, Faculty of Education Science, Universitas Muhammadiyah Surakarta, to ensure the correctness of the plant species. Fresh orange peel was washed using aquabidest and cut into smaller pieces, after which 4.0 g of it was mixed with 40 ml of aquabidest, boiled for 2 minutes, and filtered using Whatman paper No. 1 (Kaviya *et al.*, 2011). Furthermore, a silver nitrate ($AgNO_3$) solution of 1.0 mM was produced by dissolving 85 mg of silver nitrate powder into 500 ml aquabidest. The biosynthesis process was conducted by mixing 3.0 ml orange peel infusion with 40 ml of 1.0 mM $AgNO_3$, and the mixed solution was heated at 60°C for 45 minutes which was also set at room temperature for 10 minutes. The success of colloidal nanosilver form is characterized by a color change from colorless to yellowish-brown (Kaviya *et al.*, 2011).

Nanosilver characterization

The nanosilver is characterized using the instruments such as UV-Vis spectrophotometer, PSA, and SEM, which are used for monitoring the reduction from silver ions (Ag+) to nanosilver (Ag^o) with the maximum wavelength parameter that penetrates its SPR is Surface Plasmon Resonance range. After the biosynthesis process was completed and the water blanked, the samples were scanned at a wavelength range of 300–540 nm (Kaviya *et al.*, 2011; Logeswari *et al.*, 2012). Analysis was performed using PSA and SEM to determine the size and distribution of the nanosilver particles as well as show the morphology of the nanoparticles, respectively. Furthermore, the sample required for SEM analysis is a powder obtained by centrifuging the nanosilver colloidal solution at 10,000 rpm for 15 minutes. The precipitated pellet was heated using an oven at 60°C for 24 hours, and the dried nanosilver was then characterized using SEM (Kaushik and Joshi, 2015).

Nanosilver peel-off mask formulation

The amount of colloidal nanosilver as the active substance in the peel-off mask formula is 9.27 g, and it is obtained based on the minimum concentration of nanosilver as antibacterial activity. Silver nitrate used in the biosynthesis process is 170 ppm, and the silver (Ag) concentration in $AgNO_3$ obtained by comparing the relative atomic mass of Ag with molecule mass of $AgNO_3$ is 107.86 ppm. Also, the minimum effective concentration of nanosilver in cosmetic preparations is 10 mg/kg (Pulit-Prociak *et al.*, 2019). A peel-off mask of 100 g was produced to obtain a 1.0 g minimum effective concentration value of nanosilver. Furthermore, a colloidal nanosilver of 9.27 g is equivalent to 1.0 mg of nanosilver. The effective concentration of nanosilver colloid used for the peel-off mask formula is three times the minimum effective concentration.

The concentration humectant used is a modification of the preparation of peel-off mask as stated by Badnore *et al.* (2019), where PEG 400 as a humectant was 0.5%. However, this study uses a combination of glycerin and PEG 400 with a total concentration of 0.5%. First, preheat distilled water at 80°C, then dissolve the PVA powder into hot water, stir until it is melted and

Table 1. The peel-off mask formula of nanosilver using bioreductor sweet orange peel infusion with humectant combination.

Ingredients	Formula with a combination of glycerin: PEG 400 (g)				
	Base	F_1 (0:0.5)	F_2 (0.125:0.375)	F_3 (0.25:0.25)	F_4 (0.375:0.125)
Nanosilver solution	0.0	27.8	27.8	27.8	27.8
Glycerin	0.375	0.0	0.125	0.25	0.375
PEG 400	0.125	0.5	0.375	0.25	0.125
PVA	13	13	13	13	13
Phenoxyethanol	0.1	0.1	0.1	0.1	0.1
Aquadest	58.6	58.6	58.6	58.6	58.6

homogeneous, and name it mixture 1. The comparison of water and PVA used is 4:1. Whereby the PVA solution is poured into the chamber, PEG 400 and glycerin are added. Afterward, aquabidest is added to mixture 1, together with the last nanosilver colloidal, after which the mixture was stirred until homogeneous (Table 1).

Stability test of nanosilver peel-off mask using cycling test method

The cycling test was performed by storing the preparation at the temperature of 4°C for 24 hours and then storing it at 40°C for 24 hours. Before switching to a different temperature, the preparation stands at room temperature until the temperature decreases. This experiment was repeated for 6 cycles or 12 days, and the physicochemical properties of the preparation were compared before and after the experiment, although it was conducted with 3 replication.

Viscosity was measured using a Rion viscometer with spindle number 1, and the cup was filled with peel-off mask preparations while the rotor was placed in the center of the cup. After turning on the tool, wait for 1 minute and record the viscosity value (Puspitasari and Setyowati, 2019). However, the viscosity value that meets the requirement of gel preparations is 500–20,000 cPs.

The pH meter was calibrated using pH 4.01 and 6.86 buffers, and the electrode inserted into the mask preparation was then stirred until it showed a constant pH value. Afterward, the pH value was observed and recorded (Wulandari *et al.*, 2019). Therefore, the pH value that meets the skin-friendly requirement is 4.5–6.5 (Budiman *et al.*, 2017).

A dry time test was conducted by collecting 1.0 g of peel-off mask preparation and applying it to the skin over an area of 7 × 7 cm, and the time required for the mask to form a film was measured using a stopwatch (Armadany and Sirait, 2015). Meanwhile, the dry time for peel-off mask preparations is 15–30 minutes (Cahyani and Putri, 2018).

Antibacterial activity test of nanosilver peel-off mask

The equipment and materials were sterilized using an autoclave at 121°C with a pressure of 1.0 atm for 15 minutes. Also, an agar media was prepared by dissolving 8.5 g of MHA in 250 ml of distilled water (34 g/1,000 ml). Furthermore, the mixture was boiled until completely dissolved; then, it was poured into Erlenmeyer glass which was covered with cotton and then sterilized using autoclave at 121°C for 15 minutes at a pressure of 1.0 atm. MHA was poured into a Petri dish until it solidifies, and bacterial colonies were collected using a needle loop suspended in a sterile physiological NaCl solution and then homogenized. The turbidity of the measured suspension corresponds to the standard McFarland turbidity 0.5, and the bacteria suspensions were inoculated into MHA using the swabs method. Each 50 µl sample was pipetted and dropped into a well and then incubated at 37°C for 24 hours. Also, the diameter of the inhibition zone (clear area) was measured using a caliper. The clear area indicated the sensitivity of bacteria to antibiotics or other antibacterial substances, which is expressed by the width of the diameter of the inhibition zone. Tests were conducted on nanosilver solutions, peel-off mask preparations, $AgNO_3$ solutions, antibiotics, and water, and the bacterias used were *S. aureus*, *S. epidermidis*, *E. coli*, and *P. aeruginosa*.

DATA ANALYSIS

The data were analyzed using Shapiro–Wilk to determine the distribution of the data, after which it shows a normal distribution, and then a paired sample *t*-test is used to determine the significant difference of antibacterial activity, pH, viscosity, dispersion, dry time, and antibacterial test of peel-off mask preparations before and after the stability test using the cycling test method. The one-way analysis of variance was used as data for the nanosilver, in which sweet orange peel extract was used as a bioreducer. Furthermore, the statistical analysis results showed a *p*-value of <0.05, which means that it is significantly different, while if the *p*-value is >0.05, it means that the results are not significantly different. Therefore, data which data do not meet the requirements of the normality are conducted by nonparametric testing using the Wilcoxon and Kruskal-Wallis method.

RESULTS AND DISCUSSION

Plant determination was used to discover the identity of a plant to prevent sample selection errors, and this was performed using the key of plant determination. Furthermore, this was conducted at the Biology Laboratory of the Faculty of Teaching and Education Science, Universitas Muhammadiyah Surakarta. The result shows that the species of sweet orange plant used is *C. sinensis* (L.) Osbeck.

Biosynthesis process of nanosilver using sweet orange peel infusion

The active compounds that are responsible for the biosynthesis process are citric acid and flavonoid, as these two compounds contain carbonyl and hydroxyl groups as well as high concentrations in sweet orange peels (Canan *et al.*, 2016; Liew

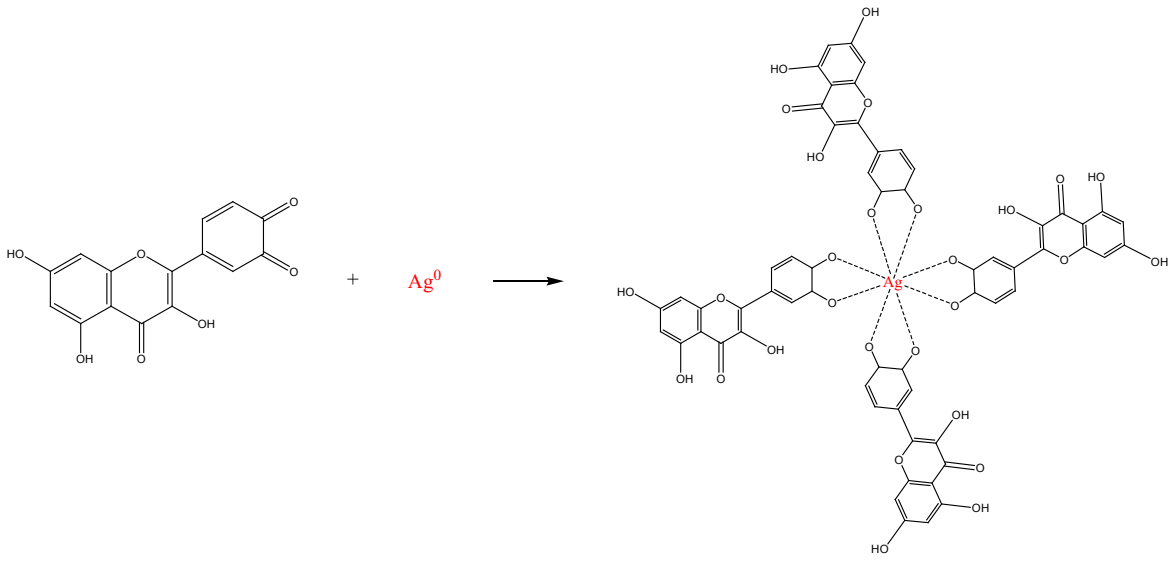

Figure 1. One possibility that can explain the mechanism of the reduction of silver ions and nanosilver chelation by the flavonoid Rutin (quercetin-3-O-rutinoside) found in sweet orange peel extract to form stable nanosilver.

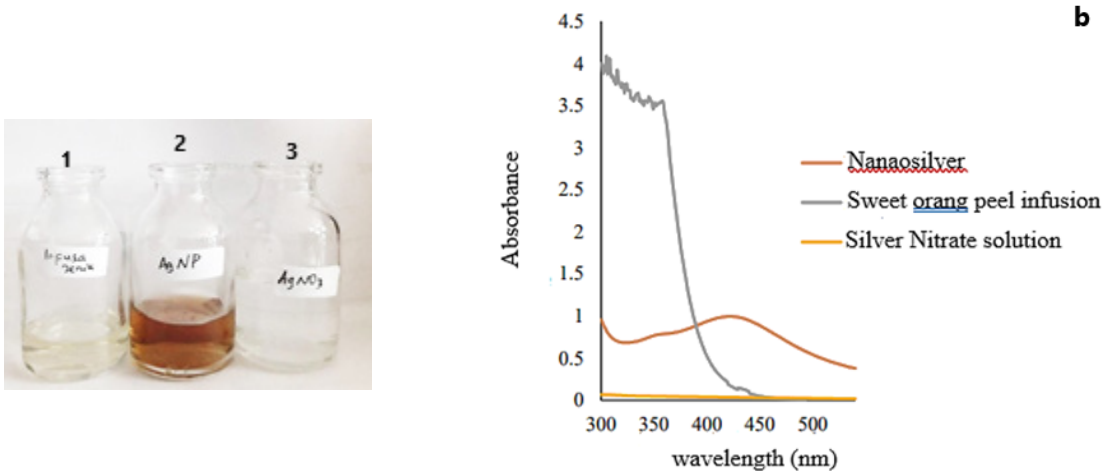

Figure 2. The results of color change to yellow-orange at seven minutes and then the color of the mixture getting dark (a). The results of scanning the wavelength of silver nitrate solution before the biosynthesis process have no absorbance at a wavelength range of 300–540 nm. Sweet orange peel infusion has many peaks in the wavelength region of 300–380 nm. After the $AgNO_3$ solution reacted with sweet orange peel infusion, a peak appears at a wavelength of 421–423 nm (b).

et al., 2018). Since flavonoids and citric acid are polar compounds, water is chosen as a solvent based on the "like dissolves like" principle of extraction (Corradini *et al.*, 2011).

Biosynthesis of silver ion by a reduction reaction, in which 1.0 mM silver nitrate solution reacts with an infusion of sweet orange peel, leads to electron transfer. A reduction reaction is a reaction caused by a substance that accepts an electron, so that charge on the atom is reduced (decrease) (Kotz *et al.*, 2010). The tautomeric transformation of flavonoid compounds from the enol to the keto, which may release reactive hydrogen, can be another mechanism in the formation of nanosilver from Ag+ ions; the mechanism can be described as follows: quercetin + 2 Ag+ ↔ quercetin +2Ag0 + 2H+ and one possibility that can explain the mechanism of the reduction of silver ions and nanosilver chelation by the flavonoid Rutin (quercetin-3-O-rutinoside) found in sweet orange peel extract to form stable nanosilver (Fig. 1). The biosynthesis process was performed at 60°C because the reaction completed faster and the resulting particle size was smaller compared to room temperature, as stated by Kaviya *et al.* (2011). In this research, the color changes to yellow-orange after seven minutes, and then the color of the mixture becomes darker. The silver nitrate solution and sweet orange peel infusion were colorless (bottle number 1,3), after the reaction process of 45 minutes, the color changes to yellowish-brown (bottle number 2) (Fig. 2). According to Ahmed *et al.* (2018), the successful formation of nanosilver from the biosynthesis process using sweet

orange peel was characterized by a color change to yellowish-brown (2). Therefore, this study showed that, after 45 minutes of reaction, nanosilver colloidal had successfully formed.

Spectrophotometry UV-Vis is a method to see the optical properties of nanosilver (Shnoudeh *et al.*, 2019), which is in the form of maximum absorbance in the SPR area. The mix solution after the biosynthesis process were scanned at a wavelength of Surface Plasmon Resonance range at 300–540 nm, where water as a blanko, and the SPR area appears in the range while water is the solvent used in the process. Meanwhile, the results of scanning the wavelength of $AgNO_3$ solution before the biosynthesis process show no absorbance at a wavelength range of 300–540 nm. This is similar to the research conducted by Wei *et al.* (2012), where the $AgNO_3$ solution shows no absorption at a wavelength of 300–700 nm. Sweet orange peel infusion has many peaks in the wavelength region of 300–380 nm; however, after its reaction with $AgNO_3$ solution, a peak appears at a wavelength of 421–423 nm with an absorbance of 0.996. This SPR range shows that silver nanoparticles have been formed (Logeswari *et al.*, 2012), and the nanosilver spectra produced are similar to that of Kaviya *et al.* (2011), namely, particle shape and maximum wavelength. The biosynthesis process of silver ions using sweet orange peel infusion at a temperature of 60°C has characteristic spectra with maximum absorption of more than 1.5 at a wavelength of 424 nm. Furthermore, the difference is in the maximum absorption, in which nanosilver spectra had an absorbance of 0.996, while Kaviya *et al.* (2011) had more than 1.5. The difference is probably caused by compounds such as citric acid and flavonoids that are responsible for the biosynthetic process and act as reducing and capping agents. However, the variation in the concentration of flavonoids and citric acid in sweet orange peel is influenced by internal factors such as plant varieties, as well as external factors, including environmental conditions, maintenance techniques, and harvest time. The phytochemical composition of sweet orange peel also is influenced by geographical conditions that affect soil type, light intensity, and humidity. The fluctuation of environmental temperature during the day and night has a significant effect on the decrease in flavonoid levels. Additionally, the duration of sun exposure to plants during the growth period correlated with flavonoid levels (Ghasemzadeh *et al.*, 2018).

The other characterization used is the PSA, which is an instrument used to determine the particle size distribution of biosynthetic nanosilver. The result of the analysis is that nanosilver has a Z-average value of 83.17 ± 7.19 nm, which shows the average value of particle distribution at Figure 4 (HORIBA, 2017). Furthermore, the process of biosynthesis showed the infusion of sweet orange peel that produces nanosilver with particle size below 100 nm. Nanosilver biosynthetic in the study by Kaviya *et al.* (2011) has a particle size of 10 ± 1 nm, and the difference in size is caused by the levels of reducing agents. The study by Prathna *et al.* (2011) showed that the reduced content of citric acid in the process of biosynthesis affects the particle size of nanosilver. Therefore, this shows that the citric acid compound is responsible for the process biosynthetic through the use of sweet orange peel infusion. The study by Osonga *et al.* (2015) showed that flavonoids are present as reducing and capping agents in nanosilver biosynthetic. Citric acid and flavonoids have a maximum wavelength of 420 and 418 nm, respectively (Hamidu *et al.*, 2018; Roukas and Kotzekidou, 2020). In this study, the absorption values at the wavelength of 418 and 420 nm are 0.667 and 0.689, respectively. However, the study by Kaviya *et al.* (2011) stated that the absorption value at the same wavelength is around 0.800, which shows that the lower the levels of citric acid and flavonoids in sweet orange peel, the bigger the particle size. The average polydisperse index obtained from this analysis is 0.522 ± 0.07; based on its interpretation, the

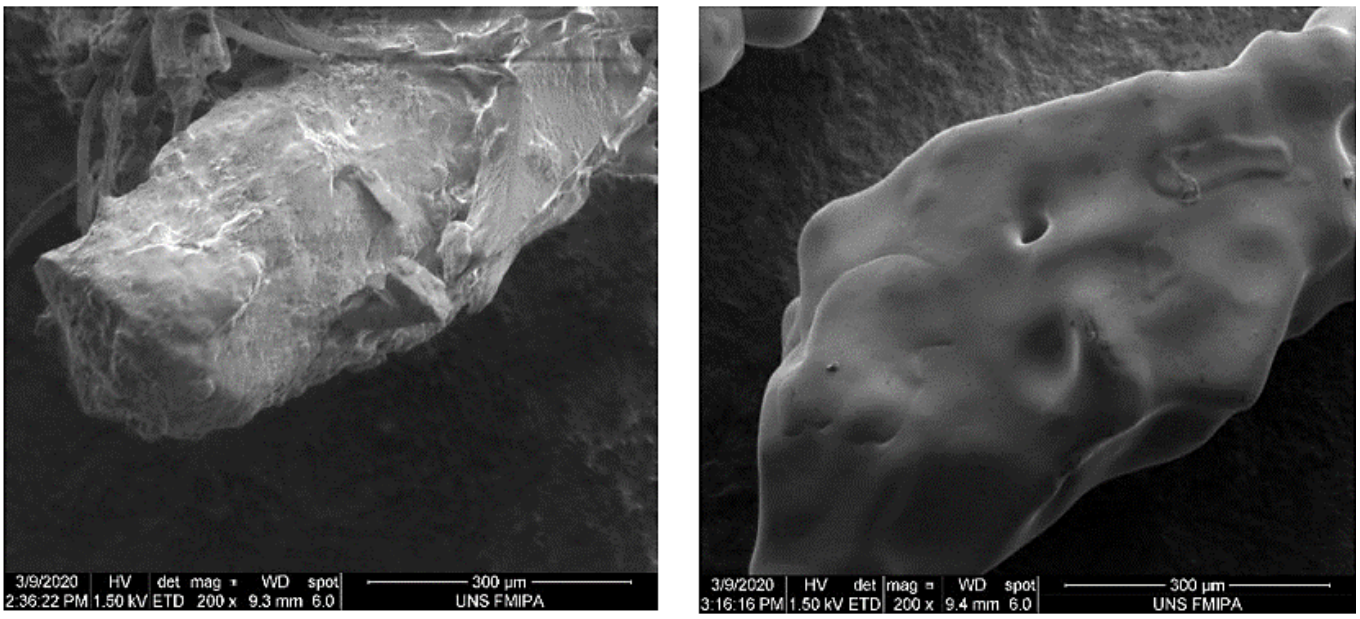

Figure 3. The results of SEM analysis. SEM represents nanosilver morphology with a magnification of 200 times. The biosynthetic process in this study produces a rod-shaped nanosilver.

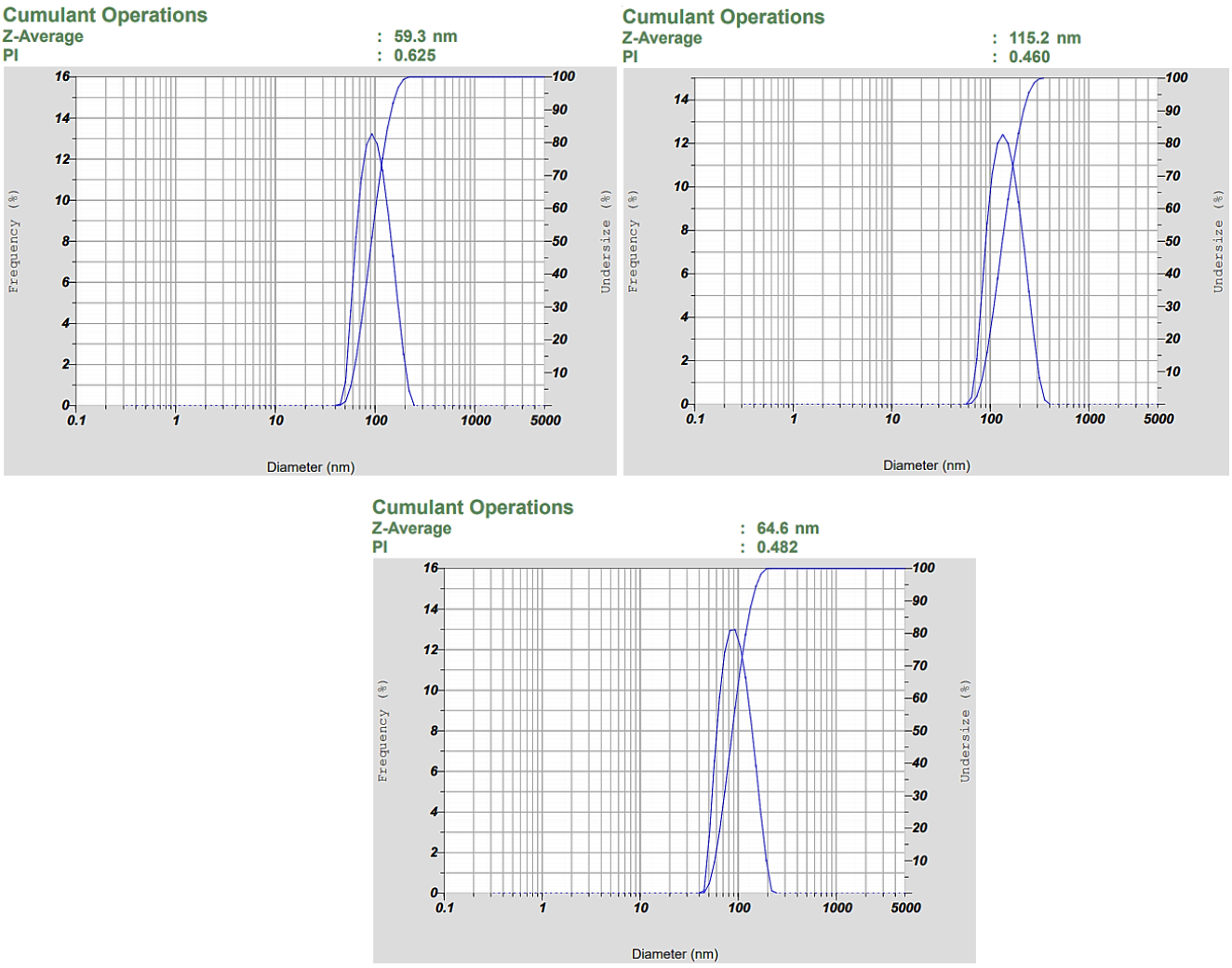

Figure 4. The result of the analysis shows that nanosilver has aZ-average value of 79.7 ± 25.19 nm and the PDI is Polidispers Index value less than 0.7, so that particles are categorized as monodisperse.

value is less than 0.7, and hence the particles are categorized as monodisperse (homogeneous dispersed) (Sreeram *et al.*, 2008). Therefore, the results of characterization using PSA showed that the biosynthesis process using sweet orange peel (*C. sinensis*) succeeded in forming a nanosilver.

Morphological characteristics of nanosilver biosynthetic using SEM instrument

In this study, the morphology of silver nitrate powder was used as a control, as SEM represents particle morphology with a magnification of 200 times. vw shows that the biosynthetic process in this study produces a rod-shaped nanosilver, and the significant difference between silver nitrate and nanosilver biosynthetic is on its surface. The particle size distribution on the silver surface is very smooth, and there are no large particles coating it. However, after the biosynthesis process, there is a biological material that coated the surface of the nanosilver formed. This material is derived from the sweet orange peel infusion that functions as a capping agent (Ibrahim, 2015).

Stability test of nanosilver peel-off mask

The total weight of glycerin and PEG 400 is 0.5 g, and both ingredients function as a humectant to maintain the viscosity of the preparation by preventing water evaporation and absorbing water from the environment. These two ingredients are combined because they have different characteristics, where glycerin is more viscous and absorbs more water than PEG 400. PVA is Polyvinyl Alcohol acts as a film-forming agent in this research, and polymers such as HPMC is Hydroxyl Propyle Methyle Cellulose and gelatin are used as film formers, but PVA has the advantage of forming good adhesive properties to provide a clean sensation. It also forms a film that is environmentally friendly and nontoxic (Kathe and Kathpalia, 2017) and interacts with humectants. Furthermore, it is influenced by moisture factors. At the low humidity, PVA forms a rigid and brittle film, but when humidity is improved, the physical properties of the film formed are soft and flexible (Ogur, 2005). The use of glycerin and PEG 400 are appropriate because they have hygroscopic properties, increasing the moisture in the preparation, and producing soft and flexible films. In addition, PVA contains adhesive after drying, which removes dirt and dead

skin cells, and when mixed with distilled water with a ratio of 4:1 and then heated at a temperature of 80°C, it breaks the inter and intramolecular hydrogen bond. The bond will break because of heat energy (Briscoe and Luckham, 2000).

Preliminary studies on the physicochemical properties of the nanosilver peel-off mask preparation were conducted for 28 days of storage at room temperature. The results showed that the difference between the concentration of glycerin-PEG 400 in the nanosilver peel-off mask formula has a significant effect on the physical properties of the preparation, such as the viscosity, dispersibility, and dry time. Peel-off mask with a composition of glycerin-PEG 400 (0.125:0.375 g and 0.375:0.125 g) has viscosity, spreadability, and pH that were not significantly different during 28 days storage at room temperature. The formula with a ratio glycerin-PEG 400 of 0.125:0.375 was selected to test for stability and antibacterial activity against Gram-positive and Gram-negative bacterias.

Viscosity describes the resistance to the flow of the preparation. A higher viscosity leads to the high flow resistance of a preparation (Gunawan *et al.*, 2012). Furthermore, the condition of viscosity value of a peel-off mask is 500–20,000 cPs, and the results show that all peel-off mask formulations meet the requirement of 500–20,000 cPs. The longer storage of the preparation decreases the viscosity value, but the value is not a significant difference. However, when the gel when is stored at high temperatures, the polymer chains formed will release the spherical roll (disentangle), hence decreasing the viscosity gel (watery). When the gel is stored at cold temperatures, the polymer chains will shorten and join each other. For a long time, the gel shrinks (entangle), thereby resulting in a change in viscosity.

Statistical test results show that there is no significant difference in the average pH value between the formulas before and after the cycling test. In topical preparations, the pH should not be too acidic because it causes skin irritation; besides that, the pH value should not be too alkaline because it causes the skin to become dry and scaly (Draelos and Lauren, 2006). The whole formula has a pH value in the range of 4.5–6.5, so the nanosilver peel-off mask formula in this study has met the requirements of gel mask preparation. In the peel-off mask preparation after the cycling test, there was a decrease in the pH, which is due to the influence of temperature. In addition, the difference in the test materials used in the peel-off mask gel also affects the stability of the pH of preparation. Despite the decrease in pH after storage using the cycling test method, all formulations and bases were still within the required pH range (Table 2).

Dry time of peel-off masks affects the comfort of use, where the longer it takes when it is too dry, the mask preparation is increasingly uncomfortable to use. The time required for peel-off mask preparations is 15–30 minutes (Cahyani and Putri, 2018). The test results show that the dry time of all formulas after the stability test with the cycling method at a temperature of 4°C and 40°C for six cycles meets the requirement of dry time. Also, the statistical analysis results for a dry time showed that during storage at 4°C and 40°C, the dry time of a peel-off mask increased, and it is caused by a temperature that absorbs water in the preparation. Other than the addition of humectants to the preparation, the dry time is also increased, and this is because PEG 400 and glycerin have hygroscopic properties that cause an absorption mechanism of water from the environment. The dry time of the peel-off mask is influenced by viscosity, where the low viscosity value affects the dry time by increasing it (Mahyun *et al.*, 2018). Furthermore, the use of a high concentration of PVA increases the viscosity value but forms a film of better quality. Also, the peel-off mask preparation contains glycerin which is hygroscopic with a high affinity for attracting and holding water molecules and maintains stability by absorbing moisture from the environment by reducing evaporation of water from the preparation. The concentration of PVA is the important factor affecting the formation of film performance in a peel-off mask. Meanwhile, preparation at a high temperature has a long dry time because of the increased temperature, which increases the volatility of water. The function of water in gel preparations is to speed up the drying time, which then affects the preparations in the form of increased dry time (Beringhs *et al.*, 2013).

ANTIBACTERIAL ACTIVITY TEST

Nanosilver peel-off mask is used for cosmetic and therapy in the treatment of acne that is caused by bacterial infection; therefore, this test aims to determine the ability of silver nanoparticle peel-off mask preparations to inhibit the growth of Gram-positive and Gram-negative bacteria under certain conditions before and after stability test. Antibacterial activity of nanosilver biosynthetic and peel-off mask preparations were conducted against Gram-positive and Gram-negative bacteria, and the good diffusion method was chosen because it is relatively easy and practical.

Table 2. The results of stability test using cycling test method of nanosilver biosynthetic.

Formula with combination of glycerin-PEG 400	Cycling test of nanosilver peel-off mask during six cycles					
	Viscosity (cps)		pH value		Dry time (minute)	
	Before	After	Before	After	Before	After
Peel-off mask base	1.33 ± 12.47	1.27 ± 12.47	5.70 ± 0.62	5.47 ± 0.13	23.17 ± 0.37	22.82 ± 0.53
0:0.5	1.80 ± 16.33	1.73 ± 18.86	5.80 ± 0.00	5.20 ± 0.25	24.00 ± 0.92	22.81 ± 0.33
0.125:0.375	2.30 ± 29.44	2.10 ± 21.60	5.37 ± 0.33	5.17 ± 0.21	22.59 ± 1.32	22.01 ± 0.37
0.25:k0.25	1.73 ± 47.14	1.63 ± 9.43	5.60 ± 0.33	5.00 ± 0.08	23.98 ± 0.50	22.98 ± 0.84
0.375:0.125	1.50 ± 0.00	1.50 ± 0.00	5.60 ± 0.80	4.86 ± 0.05	20.32 ± 0.21	20.81 ± 0.52

All values are means ± SE; $n = 3$.

Table 3. The results of diameter of inhibition zone of nanosilver biosynthetic.

Samples test	The diameter of inhibition zone (mm)							
	Staphylococcus aureus		*Staphlococcous epidermidis*		*Escherichia coli*		*Pseudomonas aeruginosa*	
	Before	After	Before	After	Before	After	Before	After
Silver nitrate	18.30 ± 1.25	-	17.05 ± 2.57	-	20.09 ± 2.15	-	17.17 ± 1.54	-
Nanosilver	20.22 ± 0.12	-	19.46 ± 0.47	-	23.51 ± 0.36	-	17.22 ± 0.90	-
Vancomycine®	22.05 ± 0.46	-	22.37 ± 1.16	-	-	-	-	-
Imipenem®	-	-	-	-	31.20 ± 0.82	-	19.26 ± 3.95	-
Water	0.0	-	0.0	-	0.0	-	0.0	-
Peel-off mask base	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AgNP peel-off mask with Glis-PEG 400 0:0.5	12.41 ± 0.15	12.05 ± 0.21	-	-	13.11 ± 0.32	13.08 ± 0.15	-	-
AgNP peel-off mask with Glis-PEG 400 0.125:0.375	13.53 ± 0.22	11.79 ± 0.51	11.82 ± 0.19	10.93 ± 0.41	13.64 ± 0.41	13.60 ± 0.21	17.46 ± 0.17	11.08 ± 0.45
AgNP peel-off mask with Glis-PEG 400 0.25:0.25	12.73 ± 0.20	12.58 ± 0.52	11.39 ± 0.15	11.08 ± 0.25	13.33 ± 0.21	13.19 ± 0.11	16.70 ± 0.54	11.83 ± 0.51
AgNP peel-off mask with Glis-PEG 400 0.375:0.125	-	-	14.57 ± 0.51	10.37 ± 0.31	-	-	18.13 ± 0.86	12.55 ± 0.21

All values are means ± SE; $n = 3$.

The sample is in direct contact with the agar media; therefore, the inhibition zone is easily identified visually. Also, this is a clear area around the wells where bacteria are inhibited by antibacterial agents. The antibacterial test was performed for 1.0 mM silver nitrate solution, the antibiotics Vancomycin® 1% for Gram-positive bacteria, Chloramphenicol® 1% for Gram-negative, and water as a negative control. Therefore, the antibacterial activity test aimed to determine the diameter of the inhibition zone of nanosilver solutions and nanosilver using sweet orange peel as bioreduction peel-off mask preparations. Vancomycin® is chosen as a control for Gram-positive infections such as *S. aureus* and *S. epidermidis*. Furthermore, it offers a significant inhibition zone based on research by Vermeluen (2000). Imipenem® is chosen as a control for *P. aeruginosa* and *E. coli* bacteria, it is an antibiotic that has a broad spectrum that works against anaerobic and aerobic bacteria, and it is effective for Gram-positive and Gram-negative bacteria. The results showed that silver nitrate solution has a strong inhibition, while nanosilver solution and Vancomycin® 1% have a very strong category of inhibition. Meanwhile, the results of the antibacterial test against *P. aeruginosa* and *E. coli* showed that the $AgNO_3$ solution, nanosilver, and control Chloramphenicol® 1% have a very strong category of inhibition. Observations showed that antibacterial activity decreased after being formulated into a peel-off mask preparation, and it is probably due to an active substance trapped in the gel polymer; therefore, it is difficult to passively diffuse to the agar medium (Table 3). The results show that all formulas are in the moderate category in inhibiting *S. epidermidis*, *S. aureus*, *E. coli*, and *P. aeruginosa* bacteria after the accelerated stability test. However, the decreasing value of the diameter of the inhibition zone after stability testing means that the storage temperature affects the inhibitory ability of the preparation.

A higher concentration of glycerin than PEG-400 increases the effectiveness of the active substance, and as a humectant, it also helps in the penetration of substances and helps the active substances inhibit bacteria. According to, glycerin also helps in maintaining excess water evaporation excess in the preparation, which is an advantage in the hot stability test for the active substance to function. The smaller size of nanosilver increases the antibacterial activity, and this corresponds to a study by Gajbhiye and Sakharwade (2016), which stated that the smaller particle size increases the surface area as well as the effectiveness. Furthermore, the mechanism of nanosilver in inhibiting microbial growth is by binding to proteins on the cell wall membrane so that the process of cellular respiration and production does not occur. The direct contact of the affected microbes with nanosilver damages the microbial cell wall and causes the difference in diameter resulting from the inhibition zone for each bacterium.

Flavonoids in plants belong to the phenol group that is known to have antibacterial activity by inhibiting the synthesis of nucleic acids, a function of the cytoplasmic membrane, and metabolism energy. It works by denaturing proteins that cause cell metabolic activity, which is catalyzed by an enzyme. Subsequently, it forms extracellular protein complexes that dissolve with the cell wall to prevent microorganisms from adhering and invading the cells. The diameter of the inhibition zone produced by nanosilver using sweet orange peel as a bioreducer on Gram-positive bacteria is not as large as that of the inhibition zone in Gram-negative bacteria. This is necessary because antibacterial compounds in the form of organic acids have greater inhibition against Gram-negative bacteria (Ermawati *et al.*, 2020). Sweet orange peel contains a class of phenolic acid compounds, organic acids, and flavonoids (Liew *et al.*, 2018), and the difference in the structure of the bacterial cell wall Gram-positive and Gram-negative affects the sensitivity to antibacterial. The cell wall of Gram-positive bacteria consists of about 40 layers of peptidoglycan, thereby reaching 70% of the dry mass of the cell

wall, therefore making it thick and stiff. Conversely, Gram-negative bacteria have peptidoglycan, which is about 10% of the dry mass, resulting in thinner cell walls.

CONCLUSION

The sweet orange peel infusion produces nanosilver with a size of 79.7 ± 25.19 nm and SPR absorption of 421–423 nm. Different concentrations of glycerin and PEG 400 affect the physical stability of the preparation, namely viscosity and dry time. Also, the preparation of a peel-off mask with a composition of glycerin-PEG 400 (0.375:0.125 g) has stability when the pH value, viscosity, and dry time show no significant difference compared to the conditions before and after the cycling test. This has the most optimum antibacterial activity with the diameter of inhibition zone against *S. epidermidis* bacteria of 14.57 mm, *P. aeruginosa* of 18.16 mm, *S. aureus* of 20.22 ± 0.122 mm, and *E. coli* of 23.51 ± 0.36 mm. Therefore, the antibacterial activity of the nanosilver biosynthetic solution has a strong category, while the peel-off mask preparation inhibits Gram-negative and Gram-positive bacteria with a moderate category before and after the stability test using the cycling method.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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