Formulation of Silver Nanoparticles Liquid Hand Wash with Polyvinyl Alcohol as Stabilizer

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Artikel Penelitian

Abstract: *According to Global Burden of Disease reported on 2019, about 1.53 million deaths caused by infectious diseases such as pneumonia and diarrhea. Triclosan is one of the active ingredient commonly used in antibacterial soap as one way to prevent the spread of infectious disease.. However, bacteria resistance against triclosan has been reported. Silver nanoparticles (AgNP) is an alternative antibacterial that potential to be used in liquid hand wash. However AgNp tend to aggregate during storage, thus stabilizer is needed This study aims to synthesize AgNP, formulate the liquid hand wash contain AgNP with polyvinil alcohol as stabilizer, and evaluate its effectiveness against Escherichia coli, Staphylococcus aureus, and Salmonella thypi. AgNP was prepared using the chemical reduction method between silver nitrate and sodium borohydride, followed by its characterization using UV-Vis spectrophotometer, TEM, PSA, and AAS. The physical characteristic of AgNp-liquid hand wash were also evaluated. Further, the antibacterial activity of AgNP-handwash was evaluated by phenol coefficient method. The peak of UV absorption spectrum of colloidal was found at 404.2 nm indicated the presence of AgNP. Ag content in AgNP colloidal was 38.405 mg/Kg ± 0,008. The spherical shape of AgNP was observed. The AgNP size was 65.1 nm with polydispersity index value of 0.543, and zeta potential value was -22.25 mV. The obtained AgNP-hand wash met the Indonesian standard criteria and was stable for 28 days. The best phenol coefficient value was obtained at formulation with addition of 30% AgNP (0.1 for S. typhi, 0.4 for E. coli, and 0.01 for S. aureus).*

Keywords: *hand wash, silver nanoparticles, polyvinyl alcohol, antibacterial activity test*

Abstrak: Berdasarkan laporan *Global Burden Disease* pada tahun 2019, penyakit infeksi telah menyebabkan sekitar 1.5 juta kematian secara global. Pengunaan sabun untuk mencuci tangan merupakan salahsatu cara dalam mencegah penyebaran penyakit infeksi. Triclosan merupakan senyawa antimikroba yang umum terkandung dalam sabun, namun kemunculan resistensi bakteri terhadap triclosan sudah mulai dilaporkan. Perak nanopartikel atau AgNP merupakansenyawa yang berpotensi sebagai antimikroba dan dapat digunakan dalam sabun tangan cair. Namun, salahsatu kekurangan AgNP dalam sediaan adalah peluang untuk terjadinya agregasi saat penyimpanan, sehingga diperlukan penstabil yang sesuai. Penelitian ini bertujuan untuk membuat AgNP dengan metode reduksi antara perak dan sodium borohydride dengan penstabil Polyvinil alcohol, mengkarakterisasi AgNP yang terbentuk dengan UV-Vis Spektrofotometer, TEM, PSA dan AAS, kemudian memformulasikannya untuk pembuatan sabun cair, serta mengevaluasi karakteristik fisik produk akhir dan aktivitas terhadap *Escherichia coli*, *Staphylococcus aureus, and Salmonella thypi.* Hasil menunjukan bahwa ukuran AgNP yang diperoleh adalah 65.1 nm dengan polydispersity index 0.543 dan potensial zera -22.2 5mV. Sabun cair AgNP yang diformulasikan telah memenuhi standar nasional, stabil selama 28 hari, dan kandungan 30% AgNP pada formulasi memberikan nilai koefisien fenol sebesar 0.1 terhadap *S. typhi*, 0.4 terhadap *E. coli*, dan 0.01 terhadap *S. aureus.*

Kata kunci: sabun cuci tangan, perak nanopartikel, polyvinyl alcohol, antibakteri

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Introduction

Infectious diseases are the common cause of death after cardiovascular disease, globally, which mainly due to lower respiratory tract infections, diarrhea, and AIDS (Acquired Immune Deficiency Syndrome)(1). In addition, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella Typhi* infection contributed to diarrhea fatality (2-4). Most of the infectious disease easily transmitted from one individual to another or from animal to human through direct contact or even contaminated food. Thus, hygienic and sanitation condition become one of the way to prevent the transmission (5) such as handwashing which is easily-practical method to prevent infectious disease transmission in community.

Antibacterial handwash commonly contain triclosan as an antiseptic agent. However, the antibacterial ability of triclosan has been reported have similar activity with ordinary hand wash(6). one of reason caused that phenomena is the triclosan need longer times to bind with bacteria enoyl-acyl carrier protein reductase than the quick-contact in handwash condition (7). In addition, bacteria resistance such as *E. coli* and *S. Thypi* against triclosan has also been reported previously (8,9).

Silver nanoparticles (AgNP) are metal nanoparticles that well known to exhibit antiviral and antibacterial abilities (10,11). Silver nanoparticles has broad spectrum activity against bacteria with multi mechanism of action such as damaging bacterial cell walls and membrane, denaturing bacteria ribosomes, preventing the ATP production and DNA replication, inducing ROS and free radicals, suppressing respiratory enzymes and membrane perforation. To note, this anti bacteria activity has been reported effective against multi-drug-resistant bacteria, Methicillin-resistant *Streptococcus aureus* (12). However, AgNP tend to aggregate after storage which lead to activity reduction (13,14). Thus, stabilizer is needed to prevent the aggregation. Polyvinyl alcohol (PVA) known as steric stabilator for nanoparticles by forming a multilayer layer around metal nanoparticles which stabilized by hydrogen bonds (13). Therefore, The synthesize and evaluation of liquid handwash formulation contained AgNP stabilized by PVA against *S. aureus, S. typhi,* and *E. coli is reported for the first time in this study*

Materials and Methods

Materials and Instruments

Silver nitrate (Merck), polyvinyl alcohol (Sigma Aldrich), glycerin (Palmac), Hydroxypropyl Methylcellulose (HPMC) (MakingCosmetics), cocamidopropyl betaine (Evonik), Ammonium Lauryl Sulfate (ALS) (Kimia Market), citric acid (Golden Sinar Sakti), sodium benzoate (Gunacipta Multirasa), potassium hydroxide (Kimia Market), fragrance (Happy Green), nutrient agar (Merck), nutrient broth (Merck), phenol (Merck), standard soap 0.05% Triclosan (Mikie Oleo Nabati), aqua demineralization (Brataco).

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 25922, and *Salmonella typhi* (culture collection of Pharmacy faculty University of Indonesia),

AAS (Shimadzu AA 6300, Japan), PSA (Malvern Mastersizer, UK), TEM (FEI Tecnai G2 SuperTwin, USA), UV-Vis Spectrophotometer (Shimadzu UV 1800-PC, Japan) pH meter (Oakton, USA), Cole-Parmer viscometer (Cole-Parmer, Germany), pycnometer (Pyrex, France), ruler, autoclave model HL-36 (Hirayama Manufacturing Corporation, Japan).

Methods

Synthesis of AgNP with PVA as Stabilizer

Synthesis of AgNP was refer to wang et al., 2005 with some modification (15). 20 mL of $AgNO_3$ solution with a concentration of 0.005 M was prepared. Then, 10 mL of 2% PVA was added. The mixture was placed on a magnetic stirrer at 660 rpm. After that, 0.6 mL of 0.2 M $NaBH_4$ solution was added little by little until an orange-yellow color was formed. Next, stirring was continued for up to 3 hours until the silver nitrate was reduced properly. Then an orange-yellow color colloid will be formed due to the reduction of silver nitrate. Colloid was stored in a dry place and kept away from light.

AgNP Characterization

UV-Vis Spectrum Measurement

UV-Vis Absorption Spectrum of AgNP was determined by diluting silver nanoparticle sample using aqua demineralization. After that, the baseline was determined using solvent (aqua demineralization). Then the diluted sample was put into a cuvette and tested with a UV-Vis spectrophotometer at room temperature (25°C). The absorption spectrum of the prepared silver nanoparticle was determined using a UV-Vis spectrophotometer at 300 – 500 nm wavelength range.

Particle Size, Zeta Potential, and PDI Measurement

The particle size, zeta potential, and PDI of the AgNP was evaluated by image analysis software on PSA (Particle Size Analyzer). Samples were prepared by diluting 1 drop of sample in 10 mL of demineralized aqua. The results of the dilution were put into a cuvette and then analyzed using PSA at room temperature (25°C).

Ag Content Measurement of Silver Nanoparticle Colloid

Ag content measurement of silver nanoparticles was carried out using AAS (Atomic Absorption Spectrophotometer). The concentration of silver nanoparticles was determined with nitric acid solution by wet digestion method. The sample was dissolved in nitric acid until homogeneous and transferred to a measuring flask. Next, the samples were analyzed at a wavelength of 328.1 nm. The absorbance value obtained was calculated using the standard silver solution calibration curve equation to obtain the sample content.

Particle Morphology

Particle morphology was determined using TEM (Transmission Electron Microscopy). The test is carried out by dropping the sample on a cooper grid that has been coated with carbon and then dried at room temperature.

Formulation of Liquid AgNP Hand Wash

Two formulation of liquid hand wash containing AgNP, named F1 and F2, were prepared according to **Table 1**.

Evaluation of Silver Nanoparticle Liquid Hand Wash

Organoleptic Test

Organoleptic testing was carried out by observing the visual condition, color, smell and stability of hand washes at room temperature on day 0, 7, 14, 21, and 28 to see the stability of the preparation (16,17). The evaluation is refer to Indonesia National standard (SNI) for pharmaceutical product (18).

Table 1. Formulation of Antibacterial Liquid Hand Wash with Silver Nanoparticles

Materials	**F1 (%)**	**F2 (%)**
Silver nanoparticle	20	30
Glycerin	10	10
HPMC	0,3	0,3
Cocamidopropyl betaine	5	5
Sodium Benzoate	0,2	0,2
Ammonium Lauryl Sulphate	2	2
KOH	1	1
Citric Acid	1	1
Fragrance	0,2	0,2
Aqua demineralization	Ad 100	Ad 100

Note: F1 refer to Formula one, F2 refer to Formula 2 pH evaluation

Measurements were carried out using a pH meter at a temperature of 25°C±2°C (16). The instrument was calibrated in a neutral solution with a pH of 7.0 and a buffer of pH 4.0, followed by electrode rinsed with distilled water and dried. The calibrated electrode was put in the sample until a constant pH value was shown. According to SNI, the pH requirement for liquid hand wash is pH 4 to 11 (18). pH measurements were carried out on day 0, 7, 14, 21, and 28.

Viscosity Measurement

Viscosity was measured with Cole-Parmer viscometer. The sample was put into the container up to its limit mark. The spindle was paired and turned on until a constant value was obtained. Viscosity measurements were carried out on days 0, 7, 14, 21, and 28.

Foam Height Measurement

A sample of 1 gram was dissolved in 10 mL of distilled water and then put into a 100 mL measuring glass. The measuring glass was shaken for 20 seconds regularly and then the height of the foam formed was measured. After 5 minutes, the foam height was measured again (19). The criteria for good foam stability according to SNI are 13-220 mm (20). Tests were carried out on days 0, 7, 14, 21, and 28.

Density Measurement

A clean and dry pycnometer was calibrated by determining the weight of the empty pycnometer. Then, the pycnometer was filled with distilled water and then weighed again. followed by addition of sample into the pycnometer then weighed. According to SNI, a good specific gravity criterion is in the range of 1.01 – 1.10 g/mL (20).

Ag Content Measurement of Silver Nanoparticle Liquid Hand Wash

Ag content measurement was done using AAS with the same method as silver nanoparticles colloid.

Coefficient phenol of Silver Nanoparticle Liquid Hand Wash

Coefficient phenol was determined following to rideal at al, 1907 with some modification (21). First, activity of phenol as positive control against tested bacteria (*S. aureus, E. coli, and S.* typhi) were carried out by preparing 3 series of test tubes consisting of a phenol solution in a ratio of 1:40; 1:80; and 1:100. Each bacteria were then inoculated by adding 0.5 mL of 10^6 bacteria/mL in each test tube followed by recording the contact time. After a contact time of 5 minutes, 1 drop of liquid was taken with a loop, inoculated into a tube containing 5 mL of Nutrient Broth, and incubated at 37°C incubator for 24 hours. This method was repeated at 10 and 15 minutes contact. The turbidity of the test tube was observed. The phenol concentration which tube showed turbidity at 5 minutes and but clear at 10 minutes were selected. The phenol coefficient was calculated as follow:

$$\text{Coefficient Phenol} = \frac{\textit{Sample concentration which shown turbidity after 5 minutes and clear after 10 minutes incubation}}{\textit{Phenol concentration which shown turbidity after 5 minutes and clear after 10 minutes incubation}}$$

Determination of coefficient phenol of liquid handwash was performed as follow: dilution series of tubes were prepared and the standard phenol solution with adjusted dilutions as control results. In a test tube containing a standard solution of phenol, 0.5 mL of a culture of 10^6 bacteria/mL (*S. aureus* or *E. coli* or *S. typhi*) was added and then shaken until homogeneous. After a 5 minutes contacted, 1 drop of liquid was taken with a loop, inoculated into a tube containing 5 mL of Nutrient Broth, and incubated at 37°C incubator for 24 hours. This method was repeated at 10 and 15 minutes contact. Then the same treatment was carried out on the first, second, and third test solutions. Tubes that experience turbidity indicate the presence of bacterial growth and clear tubes indicate the absence of bacterial growth (22).

Furthermore, the same test was also carried out on test solutions F2, F0 (bases only), and standard hand wash which contained 0.05% Triclosan. The test was carried to each of bacteria in duplicate. Phenol coefficient value was calculated by formulation mention above. Coefficient phenol evaluation was carried out on day 0 and 28 to see the effectiveness of AgNP liquid handwash after storage.

Hasil dan Diskusi

Synthesis of Silver Nanoparticles with PVA as Stabilizer

Figure 1. Visual appearance of AgNP after 24 hours

The yellowish color of AgNP after stabilization by PVA as shown at **Figure 1**, is due to the excitation of surface plasmons on metal nanoparticles (23). The nucleation, coalescence, and the growth stage are step of AgNP formation (24). The nucleation step occurs when the first or second drops of sodium borohydride were added to a mixture of silver nitrate and PVA. In this process, there was a rapid formation of black-colored particles, those particles are the nuclei of the smallest silver nanoparticles which then combine to become larger. Followed to that the particles grow and reach a stable size which is indicated by a change in the color of the colloid to yellowish or yellow-orange (25). However, further aggregation of AgNP particle was prevented by PVA that worked as coating particles by steric stabilization mechanism, thus the yellowish color could be maintained well.

Silver Nanoparticles Characterization

UV-Vis Spectrum Measurement

Based on spectrogram shown at **Figure 2A**, respectively, the peak was obtained at a wavelength of 404.2 nm with an absorbance of 0.601 which indicated that AgNPs have been formed. As previously reported, silver nanoparticles have a unique absorption, which is found in the wavelength range of 400 to 500 nm and corelate with particles size (26,27). The peak formed at the smaller wavelengthas shown at **Figure 2A** and **Figure 2C** indicates that the particle size formed is also getting smaller (27).

Particle Morphology

The morphology and shape of the particles as showed in **Figure 2B**, were determined using TEM. Based on observation, the obtained AgNP had a spherical uniform shape. To note that nanoparticle with a spherical shape are generally more thermodynamically stable (28,29). In addition, an analysis using EDS (Energy Dispersive X-ray Spectroscopy) was also carried out to see the content of colloidal silver nanoparticles made. The results of the EDS analysis as seen in **Figure 2C**, showed that Ag has the highest spectrum. Based on the % weight of the Ag element, a value of 60.79% was obtained. This indicated that the silver content on the colloid was quite large.

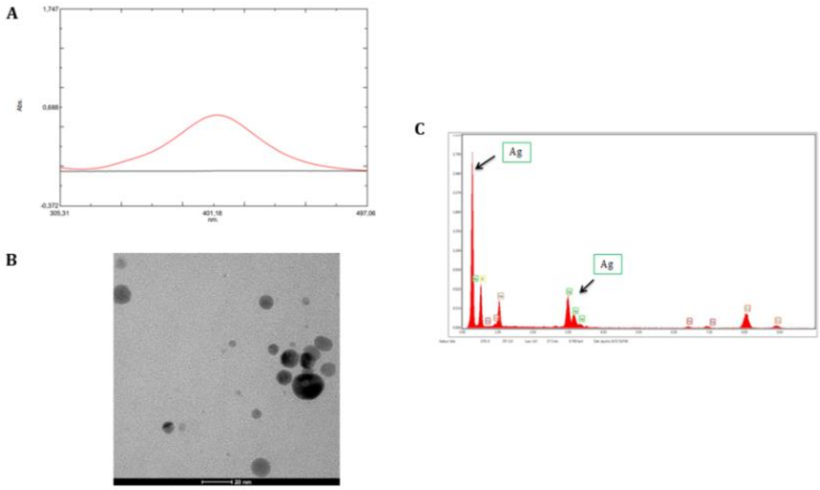

Figure 2. AgNP Characterization. A) UV-Vis Spectrum of Silver Nanoparticles; B)Morphology of Silver Nanoparticles Analyzed by TEM; C) EDS Spectrum of Silver Nanoparticles Colloid

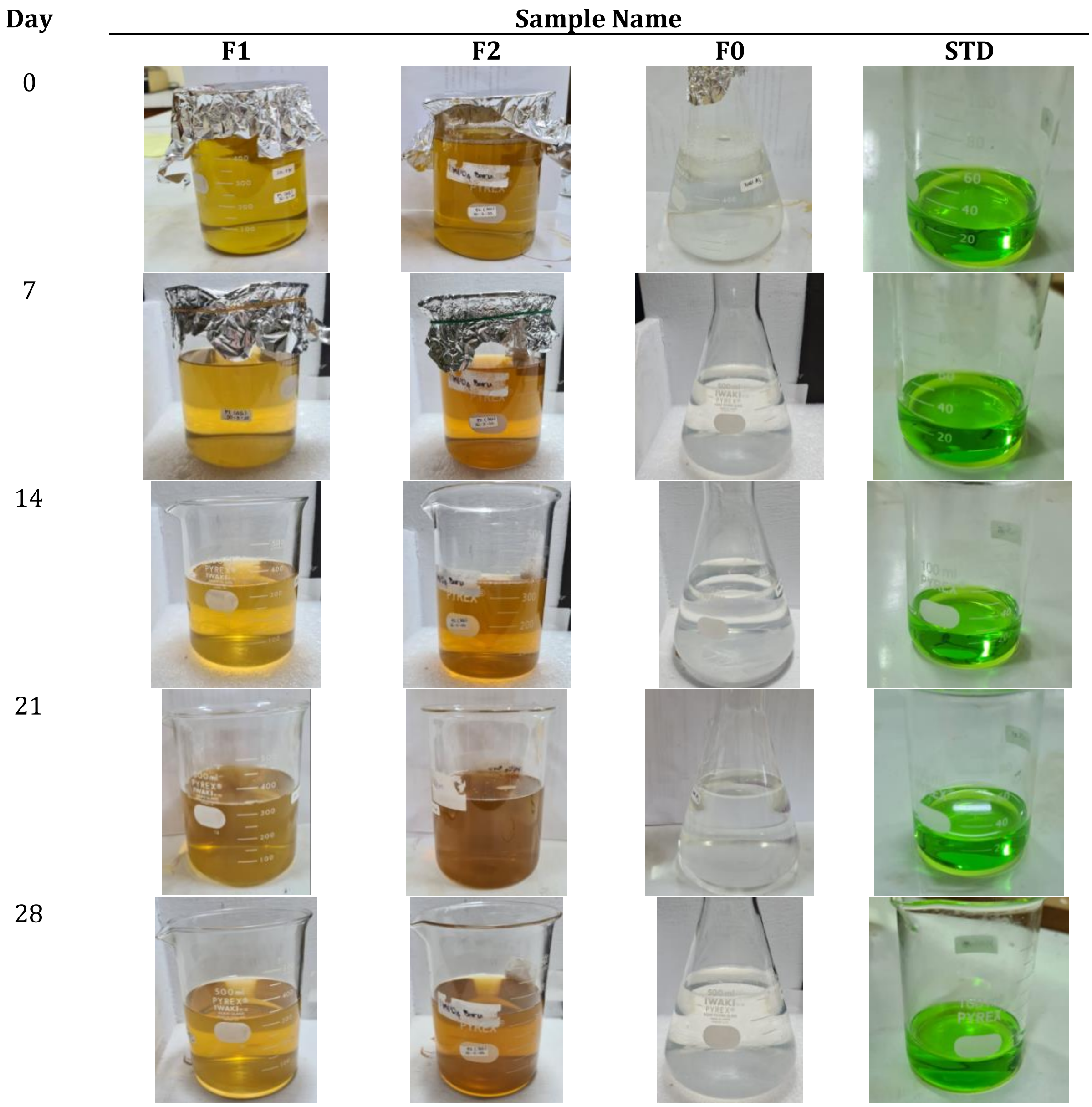

Figure 3. AgNP liquid hand wash visual characterization

Particle Size, Zeta Potential, and PDI Measurement

PSA can analyse the size and distribution of particles due to Brownian motion, a pattern of random motion of particles (28). The data obtained from PSA can be seen in **Table 2**, respectively. The average size of obtained AgNp formed was 65,1 ± 0,27 nm. This value meets the requirements for a good silver nanoparticle size which is below 100 nm (29). However, the size of the particles formed was larger than 50 nm which due to the PVA coating AgNP.

Zeta potential is a parameter used to determine the nanoparticles stability which measure the electric charge on the surface of the particles. Nanoparticles are considered to have good stability when the zeta potential value less than -30 mV or more than +30 mV. Those value indicate the nanoparticles are not prone to agglomeration (23). In opposite, zeta potential values in the range of -30 mV and +30 mV tend to cause aggregation because there is an attractive Van der Waals force (30). The potential zeta data of obtained AgNP as showed in **Table 2**, respectively was -22.25 mV which is quite close to -30 mV. Thus, the colloid formed can be concluded in stable condition. The negative zeta potential value indicated the presence of BH4- ions layer that have a negative charge (23).

The PDI values obtained was in the range of 0 to 1 as showed in **Table 2**, respectively. Particle sizes that have a PDI value less than 0.7, indicate monodisperse particles. Meanwhile, a PDI value close to 1 indicates that the particles have various sizes (polydisperse) (31). Thus, the obtained AgNP have monodisperse properties which tend to maintain their size and not experience aggregation. Therefore, they are superior to polydisperse particles, especially in optical, biomedical, and magnetic applications (32).

Ag Content of Silver Nanoparticle Colloid

Silver (Ag) content in colloidal silver nanoparticles was determined using Atomic Absorption Spectrophotometer (AAS). Results of the Ag content in colloidal silver nanoparticles made can be seen in **Table 2**, respectively. From these results, the average value of Ag content was 38.405 mg/Kg which was tend to be small because PVA stabilizers can reduce the rate of reduction of Ag^+ ions to Ag^0 (33).

Evaluation of Liquid Hand Wash with Silver Nanoparticles

In this study two formulations of liquid AgNP handwash were prepared as refered to method section, **Table 1** respectively. Thus, the evaluation of characteristic and stability need to be performed.

Organoleptic Test

Organoleptic tests were carried out at room temperature (±25°C) on two samples of liquid hand wash containing silver nanoparticles, which are F1 (Formulation with 20% colloidal silver nanoparticles) and F2 (Formulation with 30% colloidal silver nanoparticles). In addition, tests were also carried out on Bases only (F0) and standard soap with triclosan active ingredient (STD). The visual results of organoleptic testing of soap containing silver nanoparticles can be seen in **Table 3**.

Table 2. AgNP size, zeta potential and polydsipersity index

Evaluation	Value	Average
DV 90* (nm)	52,6	65,1 ± 0,270
	77,5	
Zeta Potential (mV)	-24,4	- 22,25 ± 0,136
	-20,1	
Polydispersity index	0,54	0,543 ± 0,007
	0,546	
Ag Content (mg/Kg)	38,64	38,405 ± 0,008
	38,17	

Table 3. Average pH and viscosity of AgNP Liquid Hand Wash

Evaluation	Sample	Average				
		Day 0	Day 7	Day 14	Day 21	Day 28
PH	F1	9,51 ± 0,001	9,27 ± 0,001	9,13 ± 0,002	8,68 ± 0,003	8,69 ± 0,002
	F2	9,58 ± 0,001	9,40 ± 0,002	9,26 ± 0,001	8,84 ± 0,001	8,81 ± 0,002
	STD	6,54 ± 0,002	6,48 ± 0,002	6,44 ± 0,001	6,44 ± 0,002	6,48 ± 0,002
	F0	8,61 ± 0,001	8,57 ± 0,001	8,35 ± 0,001	8,38 ± 0,002	8,38 ± 0,003
Viscosity (cP)	F1	233,3 ± 0,009	202,4 ± 0,015	200,3 ± 0,014	196,2 ± 0,009	189,2 ± 0,009
	F2	234,6 ± 0,027	204,8 ± 0,010	195,5 ± 0,008	187,9 ± 0,011	183,6 ± 0,013
	STD	625,9 ± 0,005	630,7 ± 0,003	627,8 ± 0,002	626,2 ± 0,001	621,7 ± 0,002
	F0	232,2 ± 0,021	202,6 ± 0,005	195,4 ± 0,016	188,2 ± 0,016	186,1 ± 0,024
	F0	232,2 ± 0,021	202,6 ± 0,005	195,4 ± 0,016	188,2 ± 0,016	186,1 ± 0,024

Table 4. Result of Foam Height Measurement

Sample	Foam Height (cm)									
	Day 0		Day 7		Day 14		Day 21		Day 28	
	t0	t5	t0	t5	t0	t5	t0	t5	t0	t5
F0	1,5	1,6	1,4	1,6	1,5	1,2	1,5	1,4	1,5	1,4
F1	1,5	1,4	1,6	1,5	1,5	1,4	1,6	1,6	1,5	1,5
F2	1,4	1,2	1,5	1,4	1,3	1,3	1,6	1,5	1,6	1,5
STD	1,5	1,5	1,6	1,6	1,5	1,4	1,5	1,4	1,5	1,4

Table 5. Average Density and Ag Content of AgNP Liquid Hand Wash

Sample	Average Density (g/mL)	Average Ag content (mg/Kg)
F1	1,0381 ± 0,0001	4,80 ± 0,004
F2	1,0401 ± 0,0007	9,62 ± 0,005
F0	1,0357 ± 0,0001	-
STD	1,0144 ± 0,0006	-

Based on the test results, F1 soap has a yellowish color due to the addition of colloidal silver nanoparticles. On the otherhand F2 soap was brownish yellow because more colloidal silver nanoparticles were added. The standard soap used was green due to the addition of green dye. Based on the observations made, the soap looks transparent and all the ingredients were well mixed. Based on organoleptic testing conducted for 28 days, all samples did not change color and shape. Therefore, all hand wash samples were physically stable during 28 days of storage.

pH Measurements

Based on the pH test results on **Table 3**, the pH of F1 and F2 soaps have higher pH than the blank. This was because silver nanoparticles have a pH that tends to be alkaline thus F2 preparations that have higher levels of silver nanoparticles have a higher pH, which is in the range of 7-11 (33). Storage for 28 days resulted in pH decrease of F0, F1, and F2. This due to other ingredients that had an acidic pH, such as glycerin [33]. After storage for 21 and 28 days, the pH obtained has reached stability. To note, the obtained pH of prepared liquid AgNP handwash was meet the SNI criteria (18).

Viscosity Measurements

Based on the results of the viscosity measurement that can be seen in **Table 4**, it can be seen that after 28 days of storage, there was a decrease in the viscosity of the liquid hand wash samples containing silver nanoparticles and blanks. This can occur because of the presence of glycerin which functions as a humectant that has hygroscopic properties (35). This allows absorption of moisture so the water content in the preparation increases. However, viscosity is not one of the requirements for proper soap according to SNI, but the thickness of the soap will affects consumers' interest.

Foam Height Measurement

Foam height test was indicated that all the prepared formulas could maintain the foam well after 5 minutes as seen in Table 4, respectively. The foam resistance of all prepared formula is also similar to standard hand wash and meets the requirementsof SNI, which is in the range of 13-220 mm (20). In addition, it can be seen that the addition of silver nanoparticles did not affect the height of the foam formed as evaluation at day 28 showed the foam height remained good.

Density Measurement

Based on the results in **Table 5**, it can be seen that F2 has the highest density followed by F1, and blank. This difference occurs due to the additional weight of the silver nanoparticles, which is about 10 g/mL (36). Density of samples made was above the standard hand wash, however it meet the density requirements of SNI, which were in the range of 1.01 – 1.10 g/mL (20).

Ag Content Measurement of Liquid Hand Wash with Silver Nanoparticles

The average of Ag concentration in F1 was 4.80 mg/Kg and F2 was 9.62 mg/Kg (**Table 5** respectively). The levels obtained correspond to the number of AgNP added to each formulation (F1 was 20% and F2 was 30%), respectively of the total hand wash formulation. Ag levels obtained in liquid hand soap are small because the Ag levels in colloidal silver nanoparticles made are also small, namely 38,405 mg/Kg.

Antibacterial Activity Test of Liquid Hand Wash with AgNP

Phenol Activity Test

The phenol coefficient value of phenol was different against tested bacterias as shown at **Table 6**, respectively. This is due to *Staphylococcus aureus* is a gram-positive bacterium with a thicker peptidoglycan layer than *Salmonella typhi* and *Escherichia coli* which are gram-negative bacteria. Phenol has several mechanisms of action as antibacterial, such as damaging bacteria's external membrane, reacting on bacterial wall (which distinguishes its action on gram-positive and negative bacteria), and increasing the affinity of cytoplasmic membrane by binding to membrane proteins or through slower passive diffusion which causes leakage in cells (37).

Liquid Hand Wash with Silver Nanoparticles Activity Test

Based on the value of the phenol coefficient obtained in **Table 7**, it can be seen that the phenol coefficient of F0 cannot be determined because it didn't have antibacterial ability within 15 minutes of contact time. This proves that the addition of preservatives does not affect the antibacterial activity of the soap in a fast contact time. The phenol coefficient value of the standard soap and soap samples showed coefficient value less than 1 which indicate the bactericidal ability of the standard soap and soap samples was smaller than that of phenol (38). To note, it was observed that the antibacterial ability of F2 was better than F1. This due to F2 has higher content of silver nanoparticles (30%, **Table 1** respectively). However, F1 and F2 had the same antibacterial ability against *S. aureus*. This is because *S. aureus* has thick layer of peptidoglycan so it is more difficult for silver nanoparticles to enter through the bacterial surface. The small phenol coefficient value compared to standard hand wash can be caused by the small silver nanoparticle content, which were 4.8 ppm for F1 (20% in formula) and 9.6 ppm for F2 (30% in formula).

In *Salmonella typhi*, the phenol coefficient value were 0.01 for F1 and 0.1 for F2 and standard hand wash. Then on *Escherichia coli*, the coefficient phenol values obtained were 0.1 for F1, 0.4 for F2, and 0.2 for standard hand wash. This is in accordance to previous research conducted by Qing et al., in 2018 that silver nanoparticles have better antibacterial activity against gram-negative bacteria due to thinner cell walls making them easier to penetrate.

Table 6. Coefficient Phenol Result on Positive Control

Bacteria	Ratio	Contact Time		
		5”	10”	15”
Staphylococcus aureus	1:40	+	-	-
	0.09722	+	+	+
	0.11111	+	+	+
Salmonella typhi	1:40	-	+	+
	1:80	+	+	+
	0.11111	+	-	-
Escherichia coli	1:40	-	+	+
	1:80	+	+	+
	0.11111	+	-	-

Notes: (+) means turbid (bacteria grow), (-) means clear (bacteria don't grow)

Table 7. Coefficient Phenol Value of Samples

Bacteria	Sample	Coefficient Phenol Value	
		Day 0	Day 28
Staphylococcus aureus	F0	-	-
	F1	0,025	0,025
	F2	0,025	0,025
	Standard	0,5	0,5
Salmonella typhi	F0	-	-
	F1	0,01	0,01
	F2	0,1	0,1
	Standard	0,1	0,1
Escherichia coli	F0	-	-
	F1	0,1	0,1
	F2	0,4	0,4
	Standard	0,2	0,2

In addition, the antibacterial ability of silver nanoparticles against *S. typhi* was smaller than that of *E. coli* which is also in accordance with previous research (39).

Against *E.coli*, F2 hand wash has higher antibacterial ability compared to standard hand wash because triclosan has a fairly specific antibacterial ability, namely the fatty acid synthesis process from bacteria where triclosan will bind enoyl-ACP (-acyl carrier protein) reductase and 3-oxoacyl-acyl-carrier protein synthase I and II which disrupt lipid biosynthesis (40). However, triclosan was more effective against *Staphylococcus aureus* because it may affect its regulatory system. Coefficient phenol evaluation on the 28th day, resulted in the same coefficient phenol value of F0, F1, F2, and standard hand wash. Those results indicated the stable antiseptic activity of formulated loquid AgNp handwash after 28 days of storage.

Conclusion

Silver nanoparticles with PVA stabilizer have good characteristics, including having particle size around 65.1 nm, UV absorption spectrum at wavelength 404.2 nm, a polydispersity index value of 0.543, zeta potential value of -22.25 mV, spherical form, and Ag content of 38.405 mg/Kg. The liquid hand wash formulations made, F1 and F2 are stable after 28 days of storage and meet the criteria for hand wash according to SNI. Therefore, silver nanoparticles with PVA stabilizer can be used as an alternative agent for antibacterial liquid hand wash, especially F2 with 30% colloidal silver nanoparticles (equal to 9.6 ppm AgNP) because it has better antibacterial activity, especially in gram-negative bacteria with a phenol coefficient value of 0, 1 against *S.typhi* and 0.4 against *E.coli*, and 0.01 against *S. aureus*.

Author's Contribution

All authors have contributed according to their duties and responsibilities.

Conflict of Interest

No conflict of interests has been declared by all authors.

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