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Research Article

Development of an Antimicrobial Gel Formulation for Topical Delivery Using Silver Nanoparticle

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ABSTRACT

Nanosilver is an antibacterial agent that can inhibit the growth of gram-positive bacteria such as *Staphylococcus aureus*. The small size makes nanosilver enhance its antibacterial properties. Nanosilver can be used as an active ingredient in topical preparations such as gels. In this study, the aim was to formulate and determine the antibacterial activity of gel nanosilver against *Staphylococcus aureus*. Synthesis nanosilver uses a reduction method with reducing sodium citrate and gelatin stabilizer. The Nanosilver synthesized was then characterized by UV-Vis and spectrophotometers Particle Size Analyzer (PSA). Gel preparations were nanosilver made from carbopol 940 with various concentrations of nanosilver which were 1.5%, 2%, and 2.5% which were physicochemically evaluated including organoleptic, pH, homogeneity, dispersion, viscosity, centrifugation, and cycling test at 4^{o}C and 40^{o}C. The antibacterial activity test was carried out by the excellent diffusion method using Nutrient Agar (NA) solid media with an incubation time of 1x24 hours at 37^{o}C. The test bacteria used was *Staphylococcus aureus*. Based on the data obtained showed that the gel preparation nanosilver had physicochemical characteristics which were both organoleptically clear transparent, odorless, semisolid form, had a pH range of 5.63-5.76, homogeneous, spread power 3.3-3.83 cm, viscosity 2017-2500 cP and stocks remain stable during the period of storage and cycling test. Variations in the concentration of nanosilver in gel preparations can have an effect on inhibiting the growth of bacteria *Staphylococcus aureus* with inhibition by Formula 1 (1.5%) of 4.467 ± 0.275 mm, Formula 2 (2%) of 5.683 ± 0.475 mm, and Formula 3 (2, 5%) of 6.483 ± 0.425 mm.

INTRODUCTION

One of the causes of acne is a bacterial infection. The most severe reason of acne zits accompanied by pus is the bacteria *Staphylococcus aureus*. [1] The use of antibiotics as an acne treatment tends to experience resistance. So that the development of silver-based antibacterial which tends to lower resistance [2]. Silver can be developed in the form of nanoscale (1-100 nm) called nanosilver to improve its quality [3] because the smaller the size the higher the antibacterial effect [4]. In addition, nanosilver is a new generation of antibacterial which can kill gram-positive and gram-negative bacteria such as *Escherichia coli, Staphylococcus aureus, Bacillus subtitles* [5].

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The use of nanosilver in cosmetic preparations is 12% [3].This shows that nanosilver has not been used too much in the cosmetics field. The development of cosmetic technology is very fast in the area of a combination of cosmetics and drugs (pharmaceutical) or known as cosmeceuticals [6]. One of the products cosmeceutical is an anti-acne gel preparation. Gel preparations have the advantage of being non-sticky and also a fast evaporating development. This preparation can deliver the drug well to the skin, causing acne to dry quickly [7]. Gel preparations are easier to clean from the skin surface after use and do not contain oil which can increase the severity of acne [8].

The development of antiperspirant gel preparations with nanosilver active ingredients uses a form of carbopol 940 gel because the hydrophilic base of carbopol can accelerate the release of active substances so that its capacity

as a healer can be accelerated. This hydrophilic gel base has greater stability; better spread on the skin is easily washed with water and allows the use of hairy body parts and good drug release [9].

MATERIALS AND METHODS

Material

Silver nitrate ($AgNO_3$), sodium citrate ($Na_3C_6H_5O_7$), gelatin, glycerin, carbopol 940, Triethanolamine (TEA), Aquadest, Sterile Aquadest, Solution 7, *Beef and Pepton, Nutrient Agar* (NA), Cultivate pure bacteria *Staphylococcus aureus.*

Synthesis and Characterization of Nanosilver

The synthesis was obtained by reacting 8 mL $AgnO_3$ 10^{-2}M, 0.5 mL 0.5% gelatin, 0.5 mL Sodium Citrate 3%, then added 11.5 mL sterile aquadest, then the mixture was heated using a hotplate with temperature of 100°C for 1-2 hours until the solution is brownish yellow (stable). Then the mixture is stirred using a magnetic stirrer until it is cool.

The nanosilver colloid which was finished was characterized by UV-Vis and spectrophotometers Particle Size Analyzer (PSA).

Preparation of Nanosilver Gel

The design of gel preparations nanosilver as anti-acne will be made as much as 50 grams/formula with three replications where the concentration can be seen in Table 1.

Table 1: Design of Gel Preparation Formula Nanosilver for Anti-acne

Material	Formulation		
	F1	F2	F3
Nanosilver	1.5%	2%	2.5%
Carbopol	1%	1%	1%
Glycerin	10%	10%	10%
TEA	1%	1%	1%
Aquades (ml)	86.5%	86%	85.5%

Description:
F1 = gel with concentration of nanosilver 1.5%.
F2 = gel with concentration of nanosilver 2%
F3 = gel with concentration of nanosilver 2.5%

Carbopol was dispersed into sterile aquadest and stirred slowly. Nanosilver is mixed with glycerin, then the mixture is added to the gel base that has been developed, then stirred at a speed of 1000 rpm. The final step is to add water to the desired volume, and then add TEA to reach pH 4.5-6.5 while stirring until a gel is formed.

Phytochemical Evaluation of Nanosilver Gel

Organoleptic Test

Test Organoleptic Test is done visually including shape, color, and odor. The preparation is stored at room temperature for two months. At week 0, 2, 4, 6 and 8 were evaluated.

Homogeneity test

The gel preparation was nanosilver applied between two transparent glass objects and noticed the presence of coarse particles or in homogeneity, judging by the presence or absence of particles/substances that have not been mixed homogeneously.

pH Test

Weighed the preparation of 2 g gel nanosilver then pH was measured using a calibrated pH meter. The development is stored at room temperature for two months. At week 0, 2, 4, 6, and 8 were evaluated.

Viscosity Test

Measuring the viscosity of gel preparations was nanosilver determined by a viscometer Brookfield Cone and Plate series AT71362 spindle CP-40 with rotational speed per minute (rpm) at room temperature. The tool is calibrated first. Then as much as 0.5 mL of gel is nanosilver placed in the middle of the plate; then the plate is reattached to the viscometer. A motor will drive the cone at a certain speed (rpm), and the results are awaited. The preparation is stored at room temperature for two months. At week 0 and eight an evaluation was conducted.

Spread Test

Preparation of 1 gram is placed in the middle of two round glass plates. The 125 g load is added on top of it and left for 1 minute. Note the diameter of the spread.

Centrifugation Test

The preparation was put into a centrifugation device when it was put into centrifugation at a speed of 5000 rpm for 30 minutes.

Cycling Test

The preparation was put at the temperature (4 ± 2°C) for 48 hours followed by placing the sample of the preparation at a temperature (40 ± 2°C) for 48 hours. Tests were carried out in 6 cycles and observed physical changes of gel

preparations at the beginning and end of the cycle. Preparations were made for organoleptic evaluation, pH, and the viscosity at the end of the cycling test.

Antibacterial Activity Test

Solid Media made from 5 grams of Agar Nut (NA) and 250 ml of aquadest then mixed and stirred homogeneously until boiling. Then the NA media is inserted into the petri dish. The liquid press is made from Beef and Pepton as much as 0.25 Beef and 0.15 Pepton then dissolved in 250 ml Then put into a test tube. The two media were sterilized using an autoclave at a temperature of 121^{o}C for 15 minutes with other tools.

The making of bacterial suspensions is carried out by moving one to four microbial use from pure stock into medium beef and peptone in a test tube. Then incubated at 37 ^{o}C for 24 hours and compared with McFarland's solution.

Testing with a Suitable Diffusion Method

First scraping the bacterial suspension from the rejuvenation results to the entire surface of the NA media evenly and the bottom of the cup line into four parts and then marking each formula, positive control, negative control, and similar control. After the hole is made or well with a diameter of 6 mm, the well is formed perpendicular to the surface of the media. Each well is regulated so that the observations do not overlap. Then 0.5 mg of gel added nanosilver was control of comparison, negative control, and positive control on each well that has been marked. A negative control is a gel without nanosilver. The positive control was used gel preparation benzoyl peroxide and similar control of nanosilver without gel. The media was incubated in an incubator and waited for 18-24 hours at a temperature of 36-37 ^{o}C.

Measurements were made from the bottom of the petri dish with calipers. Tests were carried out three times for each formula and then calculated the average antibacterial effect on each formula.

RESULTS AND DISCUSSION

Nanosilver Synthesis and Characterization

The process of preparation of nanosilver was 0.01 M carried out by adding drop wise sodium citrate solution to AgNOsolution$_3$ which was heated at 100^{o}C. Then stirring for 1 hour and formed brownish-yellow solution as shown in Fig. 1.

Figure 1: Colloidal Nanosilver Concentration of 0.01 M.

Chemical reaction that occurs in the reduction process using sodium citrate as the reductant is:
$4AG^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4AG^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2$

Nanosilver is formed by oxidation reduction reaction from Ag= ion from $AgNO_3$ solution. When the reduction occurs, the addition of electrons changes the Ag= ion to be not charged Ag^0 [10]. The reaction can be written based on its potential energy price so that it can know the potential cell energy, namely:

Reduction: $Ag^{+} + 1e \rightarrow Ag^{0}$ $E^0 = 0.779$
Oxidation: $4H^{+} + O_2 + 4e \rightarrow 2H_2O$ $E^0 = 1{,}224$
$E^0cell = E^0$ reduction-E^0oxidation
$E^0cell = 0.779-1.224$
$E^0cell = -0.445$

Nanosilver is characterized by UV-Vis and PSA which can be seen in Table 2. Nanosilver produces absorption peaks in the range of 427 nm which shows the formation of nanosilver. The process of forming nanosilver is characterized by the appearance of peak absorbance in the range of 400-530 nm in the Uv-Vis spectrum [11]. While the results of PSA characterization show particle size of 42.10 nm with a potential zeta of 0.02 mV. It is this small potential zeta that makes nanosilver experience a wavelength shift to 440.9 nm after 14 days of storage. Nanoparticles with potential zeta values greater than 30 mV and less than -30 mV have stable properties in suspension and can prevent aggregation of particles [12]. Agglomeration occurs because of the Van der Wals and electrostatic forces between nanosilver, namely the attraction between electrons in the nanogram, so that the distance of the nanoparticles gets closer and longer to form larger sized particles [13].

Table 2: Measurement Results Nanosilver.

Temperature	Time	Particle Size	λmax	Absorbance	Zeta Potential
100°C	1 day	42.10 nm	427 nm	0.202	0.2 mV
	14 days	Not worked	440,9 nm	0,485	Not done

Making Gel Preparation of Nanosilver

The gel made of three formulas which have concentrations 1.5%, 2%, and 2.5% where each level of formula weighs 50 grams and is replicated three times. The gelling agent used was carbopol 940. Carbopol 940 at a concentration of 0.5% -2% was able to form sufficient thickness as a gel base [14].

The production begins by dispersing carbopol 940 and aqua demineralization (academic) which are developed at a speed of 1000 rpm for 20 minutes, so that the base gel expands. Glycerin is added to the base of the carbopol 940 gel which extends and stirred at a constant speed without heating for 5 minutes. Furthermore, Nanosilver as the active ingredient is added to the gel base. Then TEA is attached to the mixture so that the pH of the preparation becomes increased or neutral according to the pH of the human skin which is in the range 4, 5-6, 5 [15]. When neutralized with TEA, the carbopol pH increases to pH 6, and in that condition, the carbopol becomes thicker. This is due to the addition of TEA, the carboxyl group from carbopol will turn into COO^-. The electrostatic resisting force between the carboxyl group that has converted into COO^- causes the carbopol to expand and become more rigid [16].

Evaluation of Nanosilver Gel

Organoleptic Test

The organoleptic evaluation was conducted to determine the physical characteristics of gel preparations including visual observations of color, shape, and odor. The results of the organoleptic test microemulsion can be seen in Table 3.

Homogeneity Test

Homogeneity examination aims to determine the homogeneity of the materials used to form the period nanosilver. Analysis of homogeneity of preparations on all three formulas gave good results, which appeared homogeneous.

pH Test

The pH test aims to determine the pH value of gel preparations nanosilver. The topical pH of the preparation should have a pH that can be tolerated not to irritate the skin, i.e. 4.5-6.5 because if the development is too acidic, it can cause skin irritation, and if the pH is too alkaline, it can cause scaly skin [17]. The three formulas show pH values that fit the pH range of human skin, which are 5.63-5.73.

Table 3: The Results of the Organoleptic Observations to Get Nanosilver.

Formula	Observation	Results
F1	Color	JT
	Form	semisolid
	Odor	TB
F2	Color	JT
	Form	Semisolid
	Odor	TB
F3	Color	JT
	Form	Semisolid
	Odor	TB

Description;
JT: Clear Transparent
TB: Not Smelling Test

Viscosity Test

The viscosity test is carried out to determine the thickness of the preparation. Viscosity is the resistance of a separate preparation to flow or spread. The viscosity of the gel preparation produced showed approximately the same viscosity. This is because the amount (%) of the gelling agent used is the same. Gel viscosity is 2240 cP, 2500 cP, and 2438 cP by F1 (1.5%), F2 (2%), and F3 (2.5%). All three formulas have good viscosity. Good gel viscosity of 2000-4000 cPs [18].

Scattering Power Test

The spread test preparation is carried out to determine the magnitude of the force required for the gel to spread to the skin or to determine the ability to spread gel preparations when applied to the surface. The semisolid spread is divided into 2, namely semi-stiff and semifluid. Semi-stiff is a semisolid preparation which has high viscosity while semifluid is a semisolid preparation with low viscosity. In the semi-stiff applied the spread power requirements were 3-5 cm^2, and for semi fluids, it was 5-7 cm^2 [19]. In gel

preparations nanosilver, the dosage form is expected to be semi-rigid, which means that the results of the spread test must be in the range 3-5 cm^2. The results of the spread of this preparation are F1 3.83 cm, F2 3.3 cm, and F3 3.53 cm.

Centrifugation Test

The results of the third centrifugation of gel formulations showed no phase separation from the gel preparation nanosilver. This means that the preparation can be said not to experience phase separation during the storage period because the centrifugation test is equivalent to the effect of gravity for one year.

Cycling Test

This test is carried out in two different temperature conditions, namely low temperature (4 ± 2 ^oC) and high temperature (40 ± 2^oC for six cycles or 12 days as a simulation of daily temperature changes to obtain gel stability information in the shortest possible time.

pH Testing after Cycling Test

For six storage cycles of gel preparations nanosilver and evaluation of pH values after stability cycling test, generally, there was a decrease in pH in each formula. However the reduction in pH was still within the pH range does not irritate the skin and the changes that occur are not significant, and it can be said that the preparation is stable. After that, a statistical analysis was performed by comparing the pH values of the developments before and after stability testing. The statistical analysis selected was One Way ANOVA, based on the test the three formulas had no difference significant because the results of the review have a significance of 0.835> 0.05.

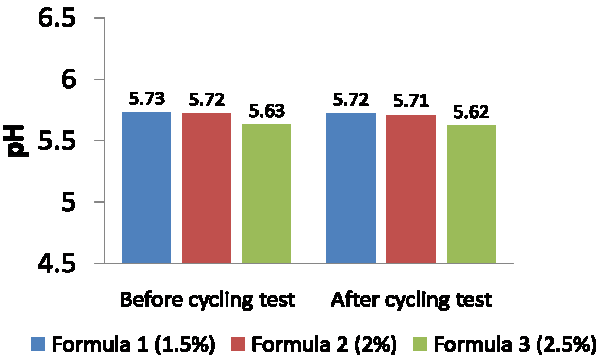

Figure 2: The Results of the Average Examination of pH Values before and after Cycling Test.

Testing of Viscosity after Cycling Test

During the six storage cycles of gel preparations nanosilver and viscosity evaluation after stability cycling test, in general, there was a decrease in viscosity in each formula. However, the reduction that occurs is still in good viscosity value. After that, a statistical analysis was performed by comparing the viscosity values of the preparations before and after stability testing. The statistical analysis chosen was One Way ANOVA, based on the test, the three formulas did not have a significant difference because the results of the review had a significance of 0.031> 0.05.

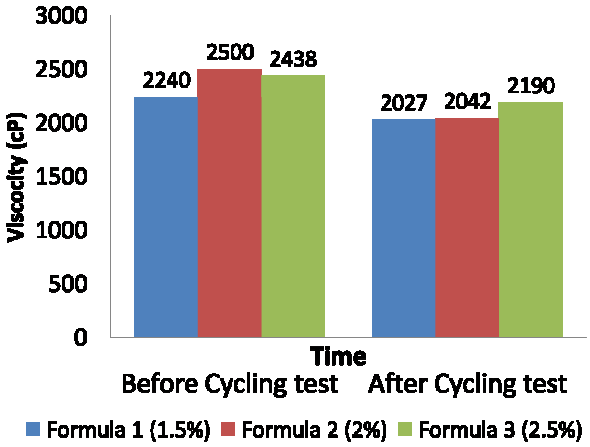

Figure 3: Results of Gel Viscosity Nanosilver after and before Cycling Test.

The Antibacterial Nanosilver Gel

Test for Testing using the good diffusion method. The first thing to do is pour the Nutrient Agar (NA) media into the petri dish and leave it stable first, then give the bacterial inoculum by scratching it to the entire surface of the press. After that, a well is made to place a sample of 4 holes per cup. Then the example was put into a well and incubated for 2 x 24 hours at 37 ° C.

Clear zones formed to determine the antibacterial ability of nanosilver in gel preparation Nanosilver against *Staphylococcus aureus* (Replication 3). The results of quantitative tests of the antibacterial ability of nanosilver in acne cream preparations measured using calipers can be shown in Table 4.

Results were analyzed with statistics One Way ANOVA. The test results at this stage show that the significance is 0,000, which means p <0.05 with a calculated F value (43,286)> F table (0.113) so it can be concluded that the null hypothesis (Ho) is rejected and hypothesis 1 (H_1)

is accepted, so the difference concentration Nanosilver has a significant effect on the value of gel nanosilver inhibitory then tested Post-Hoc which serves to see difference between groups, namely; positive controls F1, F2 and F3. In the table above shows that between F1 and F3 has a significant difference which means that the gel nanosilver at this concentration has a significant effect on the value of inhibitory bacteria *S. aureus*.

Table 4: Results of Inhibition Zone Diameter Gel Nanosilver with 6 mm Well Diameter

Concentration Nanosilver	Average ± SD
F1 (1.5%)	4.467 ± 0.275
F2 (2%)	5.683 ± 0.475
F3 (2.5%)	6.483 ± 0.425
Comparative control	9,493 ± 0.200
Controls (+)	10,683 ± 1,241
Controls (-)	0

Whereas F2 compared to F1 or F3 does not have a considerable difference. This shows that F2 does not significantly influence the inhibitory value of *S. aureus*. If the formula (F1, F2, F3) compared to the control (+) of benzoyl peroxide shows a significant difference which means that the ability to inhibit bacterial *S. aureus* is not the same because the diameter of the inhibition zone produced by positive control is greater than the inhibition zone provided by the gel nanosilver so that all formulas show results that are no better than positive controls.

CONCLUSION

The gel nanosilver preparation has evident transparent physicochemical characteristics, odorless, semisolid, homogeneous, has a pH of 5.63-5.73, the viscosity is 2240-2500 cP, and the development is stable for eight weeks of storage.

Variations in the concentration of nanosilver in gel preparations at concentrations of 1.5%, 2%, and 2.5% can affect in inhibiting the growth of bacteria *Staphylococcus aureus* shown by formula 1 (1.5%) with inhibitory power on bacteria *Staphylococcus aureus* of 4,467 ± 0.275 mm. Formula 2 (2%) of 5.683 ± 0.475 mm, and formula 3 (2.5%) of 6.483 ± 0.425 mm.

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