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ANTIFUNGAL ACTIVITY TEST OF NANOSILVER COMBINATION WITH LIP BALM COSMETIC PREPARATION

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Abstract: Nanosilveris is a silver-based material with nanometer particle sizes (1-100 nm) predicted to be antifungal or an alternative to preservatives in lip balm preparations that are biocompatible. The nanosilver synthesis process in this study used the bottom-up method by adding sodium citrate as a reducing agent and stabilizer. This study aimed to determine the characteristics of the results of 20 ppm nanosilver synthesis using a UV-Vis and TEM spectrophotometer and to determine the antifungal activity of nanosilver in lip balm cosmetic preparations. Variations in adding 20 ppm nanosilver into lip balm preparations are 5%, 10%, and 15%. The results of characterization using a UV-Vis spectrophotometer, namely 20 ppm nanosilver, were quite stable in storage for 2 months, and the results of characterization using TEM also showed that the formed nanosilver remained stable with a nanometer size range ranging from 11.42 nm to 43.95 nm. The antifungal activity test was carried out using the Direct Microscopic Count (DMC) method by counting directly under a microscope. Based on the data obtained, it shows that the antifungal activity of the combination of nanosilver and lip balm preparations is almost equivalent to the antifungal activity of parabens. It has produced lip balm preparations with nanosilver. This is indicated by the value of the inhibition rate (%). The inhibition of fungal growth produced by lip balm preparations with parabens is almost the same as the inhibition rate (%) produced by lip balm preparations with nanosilver. The inhibition rate of lip balm preparations with parabens was 89.11%. In comparison, the inhibition rate of lip balm preparations with a nanosilver concentration of 15% was 82.31%, so it can be concluded that nanosilver has good antifungal activity and is biocompatible so that nanosilver can be used as an alternative to paraben preservatives.

Keywords: *Silver Nanoparticles, Antifungal Activity, Lip Balm, UV-Vis Spectrophotometer, TEM*

INTRODUCTION

Dry skin is often a problem in Indonesia, a tropical country with high temperatures and sun exposure yearly. The lips are prone to dryness, skin cracking, and the negative effects of sunlight, such as swelling, inflammation, and color changes [1]. Cosmetics used on the lips, especially lip balms, have become society's focus in overcoming this problem. Lip balm functions to overcome dryness, moisturize, and protect the surface of the lips [1]. However, there is a risk of microbial contamination in cosmetics due to contamination of raw materials and possible contamination during use [2]. The main composition of lip balm preparations includes active ingredients, basic ingredients such as solvents, emulsifiers, preservatives, and complementary ingredients to improve quality [3]. Preservatives, such as parabens, are generally used to prevent the growth of bacteria and fungi that can damage cosmetic products [4].

Parabens are widely used as preservatives in cosmetic preparations because their use has been permitted by BPOM on the condition that the preservative added is not more than 0.4% for the single form and 0.8% for the mixed form [5]. In excess, parabens can negatively affect the body, such as toxic effects on dermatitis and skin irritation [6], and allergies can even cause cancer [7]. Thus, it is necessary to develop alternative preservatives as a substitute for parabens. This type of preservative can be developed from nanoparticle technology, which has antifungal activity in cosmetic preparations [8]. Apriliani & Aniriani's research (2017) [9], fungal contamination is microbial contamination that often occurs in cosmetic preparations.

Nano-sized materials (1-100 nm) have benefits that have been continuously developed in recent years. Silver nanoparticles have strong antifungal activity and can be used in everyday life. Their antifungal activity depends on the size of the particles, where nanosilver seeks to damage the cell walls of fungi, cell membranes, and DNA and protein synthesis, which ultimately triggers damage in cells [10]. Research conducted by Imaningsih, Junaidi, & Kurniawan (2020) showed that nanosilver can be used as an essential ingredient in cosmetics, which has antifungal properties as indicated by the results of the synthesis of AgNPs capable of inhibiting the growth of fungi in 1-3 days [11]. This can be seen from observations and calculations of colony area carried out under a microscope, showing that colloidal silver nanoparticles can cause white spores and inhibit their formation. In another study, the antifungal activity test on the morning cream preparation showed that the results of the analysis of the antifungal activity test using the disc diffusion method resulted in an inhibition zone diameter of

21.55 nm, where the administration of nanosilver in the morning cream had a very strong effect on the inhibition of Candida albicans growth [12].

Nanometers in cosmetic products are important as active ingredients, material carriers, texture stabilizers, product performance enhancers, and antimicrobial agents. Nanosilver and nanogold are effective in products such as creams, soaps, and cosmetic masks, which can fight microorganisms [13]. In cosmetic preparations, the minimum effective concentration of nanosilver is equivalent to 10 mg/kg, with a size of 20-200 nm, which only penetrates the stratum corneum as far as 2-3 μm, so it does not seep into the bloodstream. The Science Committee on Consumer Safety (SCCS) has set a limit for exposure to silver in the body so that it does not exceed 10.000 ppm [14]. Nanosilver has strong antibacterial and anti-inflammatory properties, helping to reduce the risk of infection and inflammation in dry lips. Nanosilver particles have a large surface area, increasing the penetration of active ingredients into the skin layers. Studies by [15] support that nanosilver lip balm is more effective in moisturizing and reducing the risk of infection on the lips. Nanosilver is also safe in the body, stored in the liver and other organs, with a half-life of around 50 days [16].

Setiari, Ristiati, & Warpala (2019) study showed that a combination of green bottle leaf extract and tangerine peel extract could inhibit the growth of the Candida albicans fungus with an inhibition zone of around 12.06 mm at an extract concentration of 10%;50%. However, using natural extracts has time and control constraints [17]. In contrast, research using nanosilver as an antifungal showed a larger inhibition zone, reaching 21.55 mm [12], indicating stronger potential in inhibiting fungal growth. Therefore, using nanosilver as an alternative preservative that is safer and biocompatible in cosmetics, such as lip balm preparations, has attracted researchers' attention to further research on the antifungal activity of nanosilver combinations in these cosmetic products.

RESEARCH METHODS

This study is a true experimental study. The study was conducted at the Analytical Chemistry Laboratory of Universitas Negeri Surabaya. The tools used in this study included analytical balances, glass and glassware, dropper pipettes, porcelain dishes, lip balm jar pots, spatulas, electric stove, slide glass, cover glass, universal pH, UV-Vis spectrophotometer, TEM, and optical microscope Olympus CX23. In contrast, the materials used in this study included AgNO3, distilled water, sodium citrate, cera alba/beeswax, oleum cacao, propylene glycol, castor oil, and glycerin.

Procedure for Research

1. Nanosilver synthesis

They were making nanosilver using the bottom-up method. The first step is to put 1000 ml of distilled water into a beaker. Then, the distilled water is heated until lukewarm or around 60℃. Before boiling, 20 ml of distilled water was taken, 2 grams of sodium citrate was added as a particle stabilizer, and 20 ml of AgNO3 1000 ppm. Then, heating was continued until the solution was grayish-yellow (stable). The solution formed was a 20 ppm nanosilver solution [18].

2. Characterization of Nanosilver Using a UV-Vis Spectrophotometer and TEM

The synthesized nanosilver was then characterized using a UV-Vis spectrophotometer to identify its level of stability by measuring the absorbance peak at a wavelength of 380-450 nm, with distilled water as a blank. Observations were made at week 0 and 8 with a storage time of 2 months. The Transmission Electron Microscope (TEM) characterization is used to see the morphology and size of the formed nanosilver particles [19].

3. Combination Formulation of Nanosilver with Lip Balm Cosmetic Preparations

The lip balm cosmetic preparation was mixed with the 20 ppm nanosilver using variations in the addition levels (%w/v) 5%, 10%, and 15%. The first step in making the lip balm preparation was to prepare all the ingredients, including cera alba/beeswax, oleum cacao, propylene glycol, castor oil, glycerin, and methylparaben to be weighed first using an analytical balance. Next, put the beeswax in the different cups and heat it over an electric stove until completely melted. After it is completely melted, the temperature of the electric stove is lowered. Meanwhile, in another cup, the oleum cacao is melted on the electric stove over low heat or at a temperature of around 31 - 34℃. Then, add the melted oleum cacao into the beeswax base and stir until homogeneous (Basic A).

The second stage was adding a little propylene glycol to the lip balm base mixture while stirring until it was evenly mixed at 45℃. Then, put methylparaben (especially for F1) and glycerin into another porcelain cup, then add castor oil while stirring until homogeneous (Basic B without heating). Add nanosilver to F2, F3, and F4 and homogenize again. Mix basic A and basic B. Then the cup is removed from the electric stove, put into the lip balm container, and left at room temperature until it freezes.

Table 1. Combination Formulation of Nanosilver with Lip Balm Cosmetic Preparations

Material	Formula (g)					Function
	F0	F1	F2	F3	F4	
Nanosilver	-	-	0.5	1	1.5	Active substance
Propylene glycol	0.5	0.5	0.5	0.5	0.5	Emollient
Beeswax	2.2	2.2	2.2	2.2	2.2	Hardener
Castor oil	1.5	1.5	1.5	1.5	1.5	Humectants
Parabens	-	0.02	-	-	-	Preservative
Glycerin	0.2	0.2	0.2	0.2	0.2	Emulsifier
Oleum cacao	Add 10 g	Add 10 g	Add 10 g	Add 10 g	Add 10 g	lip balm base

4. Testing for Antifungal Activity using the DMC Method

In sample preparation, negative control, positive control, NS 5%, 10%, and 15% lip balm were taken using a small spatula and placed on a glass slide. Then, 1 drop of distilled water was added before being covered with a cover glass, forming a circle on the sample in the preparation glass. This process will support testing for antifungal activity using the DMC method. Furthermore, the microscopic examination was done by taking a glass slide labeled differently, dividing it into 4 spots, and observing using an Olympus CX23 binocular microscope. Optilab Viewer 2.2 software was used to take pictures with 4x magnification, 10x objective lens, and 10x eyepiece lens. Calculating the number of fungi in the sample was carried out with the help of Image Raster software, counting the microbes that grew during 8 weeks of observation at the microscope. After the number of fungi has been identified in each lip balm sample, calculations are performed using the following formula [20]:

$$\text{Inhibition rate (\%)} : \frac{\text{number of control sample fungi} - \text{number of fungi after treatment}}{\text{number of control sample fungi}} \text{ x } 100$$

5. Testing the Physical Quality of Cosmetic Lip Balm Preparations

Testing the physical quality of lip balm cosmetic preparations involves several aspects, including:

6. Organoleptic tests

Organoleptic tests involved 15 panelists who evaluated lip balm's aroma, color, and texture.

7. pH tests

pH test questionnaires were carried out for 4 weeks using a universal pH indicator, observing the pH of the lip balm to match the range of 4.0-6.5, which corresponds to the pH of the skin of the lips [21].

Homogeneity tests

A homogeneity test was carried out using an object glass. Several samples were smeared on the object glass and then closed and pressed with another glass object, then observed homogeneity from a lip balm sample.

Skin irritation

A skin irritant test was performed on the inner forearm of 15 panelists using the open patch method, observing signs of skin irritation such as hot, itching, stings, and leather redness [22]. The whole test is important to ensure the quality and safety of lip balm preparations when used by consumers.

RESULTS AND DISCUSSION

This study aimed to determine the characterization of nanosilver synthesized using UV-Vis spectrophotometer and TEM and to determine the antifungal activity of combining nanosilver with lip balm preparations. This research was conducted in 5 stages: nanosilver synthesized, nanosilver characterization, nanosilver combination formulation with lip balm, antifungal activity test, and physical quality test of lip balm preparations.

Nanosilver Synthesis

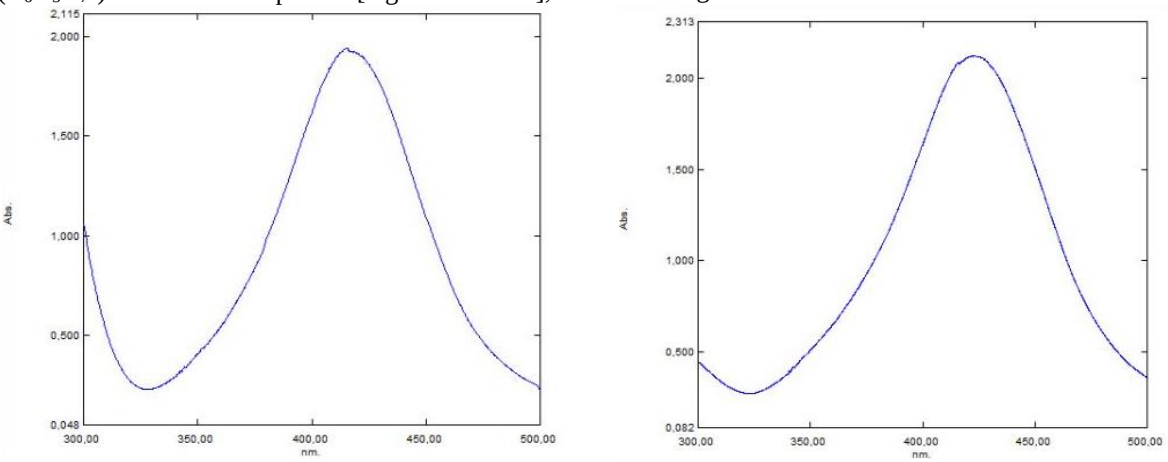
In this study, 20 ppm nanosilver was synthesized using sodium citrate as a reducing agent and $AgNO_3$ as the precursor. The synthesis process involved heating 1000 ml of distilled water to 60℃. Before boiling, 20 ml of distilled water was mixed with 2 grams of sodium citrate and 20 ml of 1000 ppm $AgNO_3$. Heating was continued until the solution reached a stable grayish-yellow color. Nanosilver 20 ppm was produced through the process.

Figure 1. Nanosilver concentration of 20 ppm

The color of the solution appears to absorption and emission of light in the visible light region through plasmon resonance or Surface Plasmon Resonance (SPR). Nanosilver synthesis results in a color change from colorless to yellow-gray, indicating the reduction of ion Ag [23]. In the early citrate stages, ion citrate reduces ion Ag+ menjadi Ag^0, forming AgNP seeds in the nucleation process. AgNP seeds complex with citrate ions ($C_6H_5O_7$-) to create complexes [Ag^{2+}- - - sitrat], aggregate to form AgNP aggregates. Ion Ag^+ is reabsorbed on the nanosilver surface and reduced by remaining citrate ions, and growth becomes stable. In synthesis, 20 ppm nanosilver, 2-gram sodium citrate is used, but usually only 0.255 gram. Excess sodium citrate forms a [cit]/$[Ag]^+$ complex with the smallest electrostatically stable silver clusters. Lack of sodium citrate produces silver clusters that do not form a feit complex [cit]/$[Ag]^+$, resulting in an incomplete reduction of Ag^+ ions [24].

Characterization of Nanosilver Using UV-Vis Spectrophotometer and TEM

In this study, to determine the stability of nanosilver Shimadzu, an 1800 UV-Vis spectrophotometer was used. The stability of the synthesized nanosilver was observed for 8 weeks of storage. Observations were made at the beginning of the 0^{th} week and the end of the eighth week. The results of the UV-Vis spectrophotometer are shown in Figure 2.



(a) (b)

Figure 2. UV-Vis Spectra of 20 ppm nanosilver at observation time (a) week-0 (b) week-8

Table 2. Results of UV-Vis spectrophotometer of silver nanoparticle test

No.	Week-	Wavelength maximum	Absorbance
1	0	415.20 nm	1.940
2	8	423.30 nm	2.125

The characterization results in Figure 2 and Table 2 show a shift in wavelength from 415.20 nm to 423.30 nm, but this shift was insignificant from week 0 to week 8. The absorption peak remained in the range of 380-450 nm, following the research of Alharbi, Alsubhi, & Felimban [25] regarding nanosilver surface plasmon resonance. The absorbance values of week 0 (1.940) and week 8 (2.125) indicate fluctuations in absorption intensity, suggesting aggregation that changes wavelength [26]. This aggregation affects the shift of LSPR (Localized Surface Plasmon Resonance) to longer and wider wavelengths, according to the concept stated by Anggraini [27]. These results indicate that the synthesized nanosilver remains stable during 2 months/8 and weeks off storage.

TEM analysis aims to determine the size and shape of the nanosilver results from uni TEM, as shown in Figure 3.

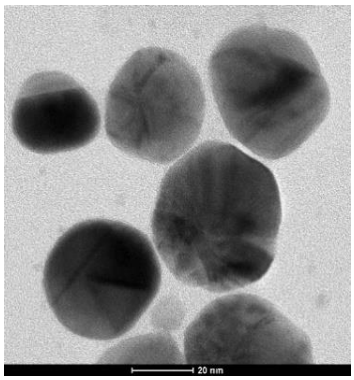


Figure 3. Characterization of 20 ppm nanosilver using TEM

The figure 3 shows that nanosilver with a scale of 20 nm has spherical and tetrahedral particle shapes with various nanosilver sizes. Namely, the smallest size is 11.42 nm, and the largest is 43.05 nm. The colors are black, gray, and white on the particles, indicating a particle's thickness. The darker the color indicates, the greater the density of the particles or the accumulation of electrons in the black part.

Excess reducing agents in solution play a role in stabilizing the formed nanosilver, so the reducing agent causes the resulting silver particles to be small [28]. This indicates that the synthesized nanosilver has a nanometer size distribution. Even though it was stored for a long time, the resulting nanosilver did not experience significant aggregation [23].

Combination Formulation of Nanosilver with Lip Balm Preparations

Lip balm is a lip moisturizer in a semi-solid dosage form with the main ingredients, namely oil, fat, and wax, which provide care to the skin of the lips [29]. In this study, 5 lip balm formulations were made with variations in the addition of nanosilver synthesis results of concentrations of 5%, 10%, and 15%, with the addition of parabens and without the addition of parabens (lip balm base). The active substance used in lip balm preparations is nanosilver, which has been synthesized by reducing sodium citrate, which acts as an antifungal. In making lip balm, propylene glycol is used as a humectant or to prevent evaporation to maintain skin moisture; glycerin is also added, which acts as an emulsifier [30]. The result of making lip balm samples is a brown water-in-oil (O/W) type preparation.

Figure 4. Lip Balm samples with various treatments

There are 5 lip balm cosmetic preparation pots containing ±10 grams each, labeled F0, F1, F2, F3, and F4. The F0 container contains lip balm cosmetic preparations without adding preservatives, resulting in a brown sample color and a semi-solid form. Container F1 contains lip balm cosmetic preparations with paraben, resulting in a brown sample color and semi-solid form. Containers F2, F3, and F4 contain lip balm preparations with the addition of 20 ppm nanosilver at concentrations of 5%, 10%, and 15%. In the F2 container, dark brown and semi-solid samples were produced, and in the F3 and F4 containers, brown and light brow samples were produced with a slightly denser shape. The greater the nanosilver content added to the sample, the color produced in the lip balm cosmetic preparation will fade (light brown) and be slightly denser in shape.

Antifungal Activity Test

Antifungal testing was carried out by the Direct Microscopis Count (DMC) method, which calculates the total microbes in a population as measured by counting directly on a microscope. In this study, observations were made every week from the first week to the 8th using a microscope to calculate the growth in the number of fungi present in each sample, which can be done with the help of Optilab viewer software and calculated using image raster software. The results of antifungal testing with the DMC method in the form of growth in the number of fungi can be seen in Figure 5.

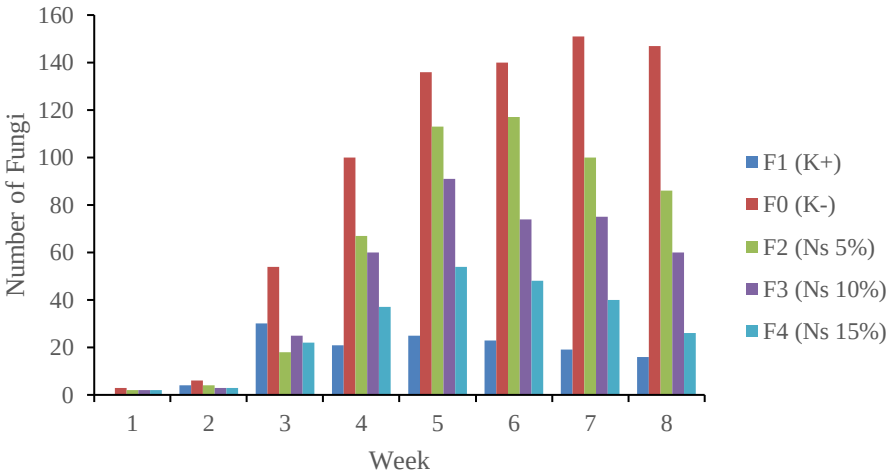


Figure 1. Graph of Growth in Number of Fungi

The figure above shows that the lip balm sample containing preservative (F1) in the third week experienced a very significant growth in fungi compared to other samples. This can happen because the amount of paraben added to the sample is only a small amount, namely 0.02 grams, given the side effects caused when used in large quantities. In addition, a decrease in antifungal activity can occur because the sample in the glass slide has direct contact with air, so the preservative undergoes oxidation [31]. After calculating the number of fungi present in each lip balm sample, the conditional determination of the inhibition rate of the fungi was carried out.

The percentage inhibition rate of the lip balm combination formulation with nanosilver on fungi growth can be seen in Table 2.

Table 3. Calculation of Inhibition Rate (%)

Sample Code	Week-							
	1	2	3	4	5	6	7	8
F1	100.00	33.33	44.44	79.00	81.61	83.57	87.41	89.11
F2	33.33	33.33	66.67	33.00	16.91	16.42	33.77	41.49
F3	33.33	50.00	53.70	40.00	33.08	47.14	50.33	59.18
F4	33.33	50.00	59.25	63.00	60.29	65.71	73.50	82.31

Based on the table 3, it can be seen that each lip balm preparation can inhibit the growth of fungi. This can be seen in Table 2. The greater the percentage of inhibition showed, the stronger the inhibitory power of the lip balm preparation samples with various variations on the growth of fungi. The F1 sample, which contained a lip balm preparation with paraben as a positive control, had the greatest percentage of inhibition compared to other lip balm preparations, namely 89.11% in the eighth week. Parabens have the property of killing microbes by being active against microorganisms. Parabens work by interfering with the work processes of mitochondria in fungi, inhibiting DNA and RNA synthesis, and destroying the structure of the lipid bilayer in fungi and bacteria so that the fungi and bacteria die.

Samples F2, F3, and F4 contained lip balm preparations added with 20 ppm nanosilver with concentrations of 5%, 10%, and 15%. From the test results, the percentage of inhibition of fungal growth was 41.49%, 59.18% and 82.31% in the eighth week. Nanosilver can inhibit the growth of fungi by attaching to all parts of the sporangiophore, conidia, and spores. This proves that nanosilver has antifungal activity. In this study, nanosilver has a good inhibition rate because, after being combined with lip balm preparations, nanosilver still has good antifungal activity and is almost equivalent to the antifungal activity of paraben preservatives. So, it can be concluded that nanosilver has good antifungal activity and is biocompatible, so it can be used as an alternative to paraben preservatives.

Table 4. Results of Organoleptic Observations of Lip Balm Preparations

Formula	Observation	Week-			
		1	2	3	4
F0 (K-)	Color	Brown	Brown	Light brown	Light brown
	Aroma	M	M	M	M
	Texture	Soft	Soft	Soft	Rather rough
F1 (K+)	Color	Brown	Brown	Brown	Brown
	Aroma	TM	TM	TM	TM
	Texture	Soft	Soft	Soft	Soft
F2	Color	Dark brown	Dark brown	Dark brown	Dark brown
	Aroma	TM	TM	TM	TM
	Texture	Soft	Soft	Soft	Soft
F3	Color	Brown	Brown	Brown	Brown
	Aroma	TM	TM	TM	TM
	Texture	Soft	Soft	Soft	Soft
F4	Color	Light brown	Light brown	Light brown	Light brown
	Aroma	TM	TM	TM	TM
	Texture	Soft	Soft	Soft	Soft

Description: M: Stinging, TM: Not Stinging

Testing the Physical Quality of Cosmetic Lip Balm Preparations

1. Organoleptic Test

This organoleptic test used 15 panelists who observed the aroma, color, and texture of lip preparations through the questionnaire sheets provide

The results of organoleptic observations for 4 weeks showed that all formulas had no change in aroma. Lip balm formula F0 without paraben and nanosilver preservative changed color to light brown in weeks 3 and 4, while the other formulas (F1, F2, F3, and F4) remained stable for 4 weeks of storage. In the F0 formula, the original texture was soft to rough after 3 weeks of storage, while the other formulas (F1, F2, F3, and F4) showed a stable texture for 4 weeks of storage. In observation after 4 weeks, the F0 formula experienced changes in color and texture, while the other formulas (F1, F2, F3, and F4) remained stable, indicating the stability of lip balm preparations.

2. pH Test

The measurement results of the five lip balm formulas follow the pH range of the lip balm skin, namely 4.0-6.5. the following data for measuring the pH of lip balm preparations are contained in Table 4 as follows

Table 5. Results of pH Test

Sample Type	Week-				Average
	1	2	3	4	
F0	5	5	5	5	5.0
F1	6	5	5	5	5.2
F2	5	5	5	6	5.2
F3	5	5	6	5	5.2
F4	5	5	5	5	5.0

Based on Table 4 above, during the storage period of 4 weeks at room temperature, the lip balm preparation did not significantly change in pH value. Tests were carried out every 1 week, and a pH value of 5-6 was produced well and followed the skin's pH range [21].

3. Homogeneity Test

The homogeneity test of lip balm preparations aims to see the presence or absence of coarse particles. The presence of coarse particles indicates that the lip balm preparations are not homogeneous because the components of the lip balm are not dispersed to form a homogeneous composition.

Based on Table 6, the results of the homogenity test showed that all lip balm preparations did not show any coarse particles when applied to the glass object. This indicates that the five lip balm preparation formulas has a homogeneous structure. A homogeneous lip balm will provide optimal results because all the ingredients are evenly dispersed in the base ingredients, so the use of lip balm will effectively protect the lips [32].

Table 6. Results of the Homogeneity Test

Sample Type	Homogeneity
F0 (Control -)	Homogeneous
F1 (Control +)	Homogeneous
F2 (Nanosilver 5%)	Homogeneous
F3 (Nanosilver 10%)	Homogeneous
F4 (Nanosilver 15%)	Homogeneous

4. Skin Irritation Test

This test was carried out on 15 panelists who were carried out by applying a lip balm formula to the inner forearm [33].

Table 7. Results of Skin Irritation Test

Sample Type	Symptoms caused			
	It is hot	Itching	Stings	Leather Redness
F0	-	-	-	-
F1	-	-	-	-
F2	-	-	-	-
F3	-	-	-	-
F4	-	-	-	-

The data shown in Table 6 shows the results of the irritation test of the five lip balm formulas that have been carried out. The panelists did not show any symptoms of irritation, such as the skin feeling hot, itchy, stings, or reddish, because the five lip balm formulas have a pH value of around 5-6, which corresponds to the pH of the skin, which is 4.0-6.5. Hence, it is safe to use and does not irritate.

CONCLUSION

This study concluded that the characterization of 20 ppm nanosilver using a UV-Vis spectrophotometer revealed stable wavelengths in the 0^{th} and eighth week. In contrast, the TEM characterization results showed variations in the size of the nanosilver with spherical and tetrahedral cluster shapes. Nanosilver still has good antifungal activity and is almost equivalent to the antifungal activity of paraben preservatives, as evidenced by the value of the inhibition rate (%) in lip balm preparations with parabens (89.11%) and nanosilver concentration of 15% (82.31%), so that nanosilver can be used as an alternative to paraben preservatives.

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REFERENCES

[1] Kadu, M., Vishwasrao, S., & Singh, S. (2015). Review Article on Natural Lip Balm—*International Journal of Research in Cosmetic Science*.

[2] Budecka, A., & Kunicka-Styczyńska, A. (2014). Microbiological contaminants in cosmetics - isolation and characterization. *Biotechnology and Food Sciences Research Article Biotechnol Food Sci*, *78*(1), 15–23.

[3] Taufikurohmah, T., & Rusmini. (2016). *Kimia kosmetik*. Jurusan Kimia Universitas Negeri Surabaya.

[4] Novita, & Ade Maria Ulfa. (2017). Penetapan Kadar Nipagin Pada Sediaan Pelembab Wajah Secara Kromatografi Lapis Tipis Dan Spektofotometri Uv. *Jurnal Analisis Farmasi*, *2*(3), 181–187.

[5] BPOM. (2011). *Persyaratan Teknis Badan Kosmetik, Keputusan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia*. BPOM RI.

[6] Susanti, H. E., Ulfa, A. M., & Purnama, R. C. (2018). Penetapan Kadar Nipagin (Methylparaben) Pada Sabun Mandi Cair Secara Spektofotometri Uv-Vis. In *Jurnal Farmasi Malahayati* (Vol. 1, Issue 1, pp. 31–36).

[7] Tjiang, W. M., Diah, N. P., Dewi, K., Agus, P., Prayoga, A., Putu, D., Suariyani, A., Kopang Maharani, G. A., Rismayani, P. A., Made, N., & Astuti, W. (2019). Analisis Kualitatif Dan Kuantitatif Kandungan Paraben Dalam Kosmetik Hand Body Lotion. *Indonesian Journal of Legal and Forensic Sciences*, *9*(2), 89–96.

[8] Latarissa, I. R., & Husni, P. (2018). Aplikasi Teknologi Nanopartikel pada Sediaan Kosmetik. *Farmaka*, *14*(1), 104–113.

[9] Apriliani, N. F., & Aniriani, G. W. (2017). Analisis Uji Mikrobiologi dan Logam Berat pada Scrub Berbahan Dasar Kapur Sirih. *Jurnal Ilmiah Sains*. 17(2). 126-130.

[10] Dwandaru, W. S. B., Putri, C. Z. M., & Yulianti, E. (2016). Pengaruh variasi konsentrasi bahan aditif laruutan nanopartikel perak terhadap seifat anti-jamur cat dinding sebagai aplikasi teknologi nano dalam industri cat dinding. In *Inotek* (Vol. 20, pp. 1–18). http://edupaint.com

[11] Imaningsih, W., Junaidi, A. B., & Kurniawan, I. (2020). Uji potensi antifungi koloid perak nanopartikel (AgNPs) terhadap Aspergillus niger. *Bioscientiae*, *17*(1), 25–39.

[12] Adelia Jalestri, D., & Titik Taufikurohmah. (2016). Uji Aktivitas Antifungi Nanosilver Dalam Krim Pagi Terhadap Fungi Candida albicans. *UNESA Journal of Chemistry*, *VII*(28).

[13] Isnaeni, I. W., Mahmudi, & Rifada, A. (2020). *Studi Kelayakan dan Keamanan Penggunaan Nanogold Sebagai Bahan Baku Produk Kosmetik*. Lembaga Ilmu Pengetahuan Indonesia, Kawasan Puspiptek.

[14] (SCCS), S. C. on C. S. (2018). Colloidal Silver (nano). http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm

[15] Zou, Y., Zhao, L., Zhou, Y., Huang, Y., & Liu, D. (2018). The Effects of Nanosilver-Containing Lip Balm on Lip Skin Moisturization. *Journal of Cosmetic Science*, *36*(2).

[16] WHO (2003). Silver in Drinking Water. *Journal of Guidelines for Drinking-Water Quality*, *2nd*, 2.

[17] Setiari, N. M. N., Ristiati, N. P., & Warpala, I. W. S. (2019). Aktivitas Antifungi Kombinasi Ekstrak Daun Sirih (Piper betle) dan Ekstrak Kulit Buah Jeruk (Citrus reticulata) untuk Menghambat Pertumbuhan Candida albicans. In *Jurnal Pendidikan Biologi Undiksha* (Vol. 6, Issue 2, pp. 72–82).

[18] Cahyanti, A. D. (2022). Production and Organoleptic Test of Body Sanitizer Nanogold, Nanosilver, and Eucalyptus Oil in the Pandemic Covid 19. *International Journal of Current Science Research and Review*, *05*(04), 1861–1870.

[19] Taufikurohmah, T., & Tantyani, T. A. (2020). Antibacterial and Antifungal Activity of Silver Nanoparticles Against Neisseria Gonorrhoeae and Candida Albicans. *International Journal of Research - GRANTHAALAYAH*, *8*(6), 179–187.

[20] Li, L., Pan, H., Deng, L., Qian, G., Wang, Z., Li, W., & Zhong, C. (2022). The antifungal activity and mechanism of silver nanoparticles against four pathogens causing kiwifruit post-harvest rot. In *Frontiers in Microbiology* (Vol. 13, pp. 1–16).

[21] Erwiyani, A. R., Destiani, D., & Kabelen, S. A. (2018). Pengaruh Lama Penyimpanan Terhadap Sediaan Fisik Krim Daun Alpukat (Persea Americana Mill) dan daun sirih hijau (Piper betle Linn). *Indonesian Journal of Pharmacy and Natural Product*, *1*(1).

[22] Fauziah, A. (2021). *Formulasi dan Evaluasi Fisik Sediaan Lip Balm dari Kulit Papaya*. Skripsi. Politeknik Harapan Bersama.

[23] Nurcahyo, R. Y., & Wibawa, P. J. (2022). Fabrikasi Ramah Lingkungan Komposit Nano Karbon Aktif-Partikel Perak dan Uji Aktifitas Antibakterinya. *Greensphere: Journal of Environmental Chemistry*, *2*(1), 31–37.

[24] Arfi, K., & Taufikurohmah, T. (2017). Pemanfaatan Nanosilver Sebagai Antibakteri dalam Sediaan Farmasi Krim Pelembab Mata. *UNESA Journal of Chemistry*, *6*(1), 11–19.
[25] Alharbi, N. S., Alsubhi, N. S., & Felimban, A. I. (2022). Green synthesis of silver nanoparticles using medicinal plants: Characterization and application. *Journal of Radiation Research and Applied Sciences*, *15*(3), 109–124.
[26] Aritonang, H. F., Katja, D. G., & Lolo, W. A. (2022). *Biosintesis Nanopartikel Perak (Ag) Menggunakan Ekstrak Daun Afrika (Vernonia Amygdalina) Dan Polietilen Glikol 6000 Serta Aplikasinya Sebagai Detektor Ion Logam Berat*. Universitas Sam Ratulangi Manado.
[27] Anggraini, F. N. A. (2018). Validasi Metode Analisis Melamin Menggunakan Nanopartikel Perak (NPP) Hasil Bioreduksi Ekstrak Buah Kersen (Muntingia calabura L.). *Jurusan Farmasi UIN Malang*.
[28] Apriandanu, D., Wahyuni, S., & Hadisaputro, S. (2013). Sintesis Nanopartikel Perak Menggunakan Metode Poliol Dengan Agen Stabilisator Polivinilalkohol (PVA). *Jurnal MIPA*, *36*(2), 157–168.
[29] Inawati, R., S., & Juliana, M. (2021). Formulasi dan Evaluasi Sediaan Lip Balm Ekstrak Kayu Secang (Cesalpinia sappan L Sebagai Pewarna Alami. In *Sekolah Tinggi Teknologi Industri dan Farmasi Bogor*. Sekolah Tinggi Teknologi Industri dan Farmasi Bogor.
[30] Bella Mega Silvia, & Mentari Luthfika Dewi. (2022). Studi Literatur Pengaruh Jenis dan Konsentrasi Basis terhadap Karakteristik Masker Gel Peel Off. *Jurnal Riset Farmasi*, *2*(1), 30–38.
[31] Jabal, C., 1∗, R., Pujiyanto, S., & Rukmi, I. (2021). Analisis Mikrobiologis Produk Lipstik Cair yang Digunakan oleh Penata Rias. *Journal of Biology and Applied Biology*, *4*(2), 105–114.
[32] Aidina, S. (2020). *Formula dan Aktivitas Antioksidan Sediaan Lip Balm yang Diperkaya Ekstrak Daun Bidara (Ziziphus spina-christi L .) Skripsi 2020 M / 1441 H*. Program Studi Kimia Fakultas Sains dan Teknologi Universitas Islam Negeri Syarif Hidayatullah Jakarta.
[33] Nst, R. A. (2022). *Uji Fisikokimia Pada Sediaan Lip balm Dari Minyak Pala (Myristica fragrans Houtt)* (pp. 1–60).