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# Formula Development and Characterization Testing of Niacinamide Nano Liposomes as Anti Aging

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A R T I C L E I N F O

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A B S T R A C T

Niacinamide has a mechanism of action as an anti-aging agent by increasing fibroblast production by stimulating collagen synthesis and can increase elastin which can prevent wrinkles on the skin of the facial area. The most commonly used method is thin layer hydration followed by sonication. Determination of the optimum formula using expert design with the simplex lattice design method. The purpose of the study was to determine the effect of Phosphatidylcholine concentration of 0.5-5% and Cholesterol concentration of 0.3-5% on the physical properties of niacinamide liposomes, obtain the optimal formula for nano liposomes niacinamide and determine the anti-aging activity of Niacinamide liposomes tested on the backs of rabbits. In the simplex lattice design method, the optimal formula of Phosphotidylcholine with a concentration of 4.7% and cholesterol with a concentration of 0.3% was obtained which produced nanoliposome characteristics including particle size of 135.7 zeta potential -77.19, Adsorption Efficiency of 98.01% and has anti - aging activity with parameters increasing collagen 29.98% and elasticity 65.56%. Niacinamide preparations provide a large increase in anti-aging activity and increase permeability so that they can provide a faster effect than niacinamide serum without liposomes.

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## INTRODUCTION

Niacinamideor known as vitamin B3, has an anti-aging method of action involving increased fibroblast proliferation, collagen synthesis, and elastin formation. Niacinamide, which is widely used as a cosmetic, has a safe range of 1% to 5% when applied topically for a long period of time (Bissett, 2009). When applied topically for 12 weeks, 5% niacinamide significantly improved the appearance of skin in female respondents aged 50 years or older. These improvements included reduced wrinkles and fine lines, greater elasticity, red spots and pale skin, and hyperpigmentation spots (Bissett, 2009).

In addition to its anti-aging properties, niacinamide has antibacterial, anti-inflammatory, skin brightening, and UV protection properties (Gehring, 2004). Niacinamide is a white crystalline powder or odorless and colorless crystals. Niacinamide is well soluble in ethanol and water. In addition, niacinamide is heat stable (FI VI, 2020). When prepared in topical applications, niacinamide is hydrophilic; however, the stratum corneum is a barrier to penetration (Renata Basto, 2021). The benefit of the drug delivery system is that it can penetrate the skin deeper. Nanotechnology, or the use of nanoscale particles in technology, is a recent advancement in the pharmaceutical, food, and cosmetic industries (Papakostas *et al*., 2011). Liposomes, niosomes, ethosomes, transfersomes, and phytosomes are some of the advances in drug delivery systems that can penetrate the stratum corneum (Ramadon & Mun‟im 2016).

Liposomes are very small vesicles that have a lipid-based membrane that encloses the entire volume of water. When lipids are dispersed into aqueous media, they spontaneously form liposomes, which are small vesicles with dimensions varying from tens to hundreds of nanometers. Cholesterol can be added to liposomes to increase their stability, and phospholipids, both synthetic and natural, can also cause liposome formation. Drug delivery systems for lipophilic, hydrophilic, and amphiphilic chemicals can be derived from liposomes (Hamid Reza Ahmadi Ashtiani, 2016). In order for active substances to better penetrate the stratum corneum, liposomes in cosmetic form can increase lipids, reduce water loss, and help make ingredients more soluble in the skin layer. (VD Ngoc Thuy Trang Le, 2019).

Phosphatidylcholine concentration of 0.5–10% functions as an emulsifier. Drug molecules can dissolve in lipids and pass through the stratum corneum, while phosphatidylcholine and active chemicals will form persistent liposomes (Nandure *et al*., 2013; Kidd 2009). In addition to phosphatidylcholine, liposomes also contain cholesterol. This cholesterol works by reducing fluidity, which can stop leakage, and permeability in the water-soluble liposome membrane. The role of an emulsifier can be played by cholesterol concentrations between 0.3 and 5.0% in topical or semisolid formulations (Nandure *et al*., 2013; Kidd 2009).

One of the optimization techniques that can identify the ideal composition of a mixture of materials, provided that the total amount of materials used in the formulation is constant and consists of at least two material components, is called Simplex Lattice Design(SLD). Benefit sSimplex Lattice Designis its practicality, speed, and minimal trial and error (Suryani Tambunan, TN 2018).

This technique is used to identify the optimum solvent and calculate the ideal formula for semi-solid solid preparations..

The purpose of this study was to determine the effect of Phosphatidylcholine and Cholesterol on the physical properties of niacinamide liposomes. To determine the maximum concentration of phosphatidylcholine and cholesterol using the Simplex Lattice Design method. To determine the anti-aging activity of niacinamide liposomes tested on the backs of New Zealand rabbits.

The formed liposomes were characterized by testing parameters, namely particle size, zeta potential, entrapment efficiency and anti-aging activity tests with collagen and elasticity parameters.

## LITERATURE REVIEW

### *Cosmetics*

Cosmetics are preparations that are used for external parts of the body such as: hair, lips, nails, external genital organs, teeth and oral mucosa. Aims to be able to clean, perfume, change appearance and/or improve body odor or protect or maintain the body in good condition (BPOM, 2022).

Cosmetics consist of active ingredients, fragrances, preservatives, stabilizer, lipids, water, alcohol and other dissolved materials and dyes. Cosmetic ingredients can come from nature or synthetic which can be used as cosmetic production. Cosmetic preparations must meet the requirements according to the Indonesian cosmetic codex or other recognized standards. The classification of cosmetic treatments that function to treat skin disorders is (BPOM, 2022):

Cosmeticspremature agingworks to overcome skin aging, premature aging. Cosmetics Hyper pigmentation works to treat skin disorders such as acne and black spots. Cosmetics Dandruff functions to treat scalp and hair roots, such as dandruff, oily hair (seborrhea), and excessive hair loss.

### *Skin*

The skin is a protective tissue of the body that has a flexible and elastic structure. 15% of the body is skin which is equivalent to the weight of an adult (Rostamailis 2005). The skin has the main function of providing protection for the body from external stimuli, can prevent the penetration of dangerous foreign objects, can protect against radiation rays and can maintain skin moisture.

### *Skin Aging*

With increasing age, aging will also occur in humans. The occurrence of aging, wrinkles on the skin can also cause a decrease in skin function. Based on data obtained from world population data, there has been an increase in the elderly population over 65 years of age, a significant increase, namely from 8% in 1950 to 11% in 2009, and there could be an increase of up to 20% in 2050. From these data, it was concluded that there are health problems with aging and increased aging of the skin. Skin aging affects social and individual life (Ahmad Zahruddin and Damayanti, 2018). In general, there are two factors that cause aging, namely the first factor of aging is caused by intrinsic factors or chronological aging related to increasing age. The intrinsic aging process is a

process that cannot be inhibited and this process runs slowly. The mechanism of occurrence is the occurrence of changes in the structure of the skin in the layers epidermisand the occurrence of biochemical changes that occur in the layers dermis. These changes can also be seen in skin organs such as hair and sweat glands. The characteristics of intrinsic changes are that the skin looks paler, there are fine wrinkles (fine wrinkles), the skin appears thinner, more transparent and looks more fragile in the outer layer. Epidermis Anddermis due to atrophic changes. The reaction felt by the skin is drier and itchy.

***Liposomes.***

Name liposomecomes from the Greek words 'Lipos' meaning fat and 'Soma' meaning body. Liposomare microscopic vesicles in which the water volume is completely enclosed by a membrane consisting of lipid molecules. Liposom formed spontaneously when lipids are dispersed into aqueous media, which then form vesicles with diameters ranging from tens of nanometers to hundreds of nanometers. Liposom has a polar or hydrophilic structure at the head and non-polar or hydrophobic at the tail so that it can be used in the delivery system for compounds that are lipophilic, hydrophilic and amphiphilic. Hydrophilic drugs are trapped in the water core while lipophilic drugs are trapped in the lipid bilayer (Verawaty, 2016).

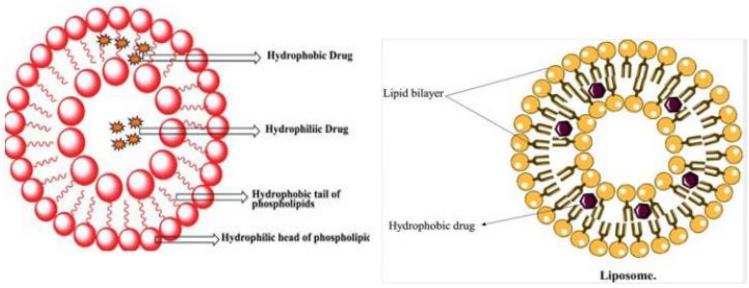



**Figure 1**. Structure Liposom

ProfitLiposomfor topical preparations is to reduce serious side effects and incompatibilities of drugs to avoid systemic absorption, can increase the effect of drugs in small doses on the skin, as a drug delivery system that is soluble in water and lipids. Liposomin cosmetic form helps improve the properties of active ingredients, can penetrate lipids and reduce water loss so that it can penetrate the stratum corneum (Hamid Reza, 2016). Liposomcan be formed because of the existence ofphospholipids natural or synthetic and the addition of cholesterol which can increase stability Liposom.

***Method Simplex Lattice Design***

Method Simplex Lattice Designis an optimization method that aims to determine the physical properties of two or more mixtures. With this method simplex lattice designt here is no need fortrial and errorin determining the optimal formula (Bolton, 1997). The principle of this method is to determine the optimal formula of a mixture of materials with the condition that the total amount of materials used in the formulation is always constant. The advantages

of Simplex Lattice Design namely a simple optimization method, can be used to optimize the mixture of solid, semi-solid dosage forms, or solvent selection.SLD operated by software called design expert. General equation Simplex Lattice Design for two factorial.

## METHODOLOGY

### *Research Tools and Materials*

UV-Vis Spectrophotometry, rotary evaporator (Heidolph), probe sonicator (QSonica, Newtown, USA), magnetic stirrer (Thermo Scientific, China), centrifuge (SPLC Series, Gemmy 8 Hole, Taiwan), particle size analyzer (Malvern 3000E), analytical balance (Ohaus), glassware (Pyrex, Japan), non-glassware available in the laboratory and skin analyzer.

The materials in this study wereNiacinamide (Mahachem), Phosphatidylcholine (Sigma-Aldrich), Cholesterol (PT. Tirta Buana Kemindo), ethanol (Brand), chloroform (Brand), distilled water (Brand), Phosphate Buffer Saline (Brand).

### *Research flow*

### *Formula Optimization Experiment Design*

Using two variables of liposome components phosphotidylcholine, with a concentration range of 0.5–5%, and cholesterol, with a concentration range of 0.3–5% at low and high levels, the formula was optimized using the Design Expert® program version 13 with the simplex lattice design method. The desired response was measured based on particle size, zeta potential, entrapment efficiency, and anti-aging activity test with collagen and elasticity parameters. The findings were obtained up to 10 times of formula testing. Table 1.

**Tabel 1. Formula *Liposom* Niacinamde dalam % (*SLD*)**

| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Niacinamide | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Fosfotidilkolin | 3.3 | 1.9 | 0.5 | 4.7 | 3.65 | 4.7 | 2.6 | 4.7 | 1.55 | 0.5 |
| Kolesterol | 1.7 | 3.1 | 4.5 | 0.3 | 1.35 | 0.3 | 2.4 | 0.3 | 3.45 | 4.5 |

### *Liposome manufacturing*

In an Erlenmeyer flask, 10 ml of 96% ethanol was added to niacinamide and stirred until dissolved, 10 ml of 96% ethanol was used to dissolve phosphatidylcholine, which was then added to an Erlenmeyer flask containing niacinamide solution, 10 ml of chloroform was used to dissolve cholesterol, which was then stirred until completely dissolved in the Erlenmeyer flask. The solvent used was a mixture of niacinamide and phosphatidylcholine solutions in a ratio of 1:1.For ten minutes, use a magnetic stirrer to mix the solution mixture thoroughly. After the mixture becomes uniform, transfer it into a round-bottomed flask and use a rotary evaporator set at 50 °C and 50 rpm to remove the lipid from the solvent and precipitate it in a thin layer on the flask wall. To hydrate

the lipid, add 20 ml of pH 7.4 phosphate buffer solution to the lipid layer. After sonication, the product is a multilamellar vesicle that can maximize drug absorption and has a smaller particle size (Babazadeh *et al*., 2018). Sonication. This approach works by adding 20 ml of phosphate buffer solution of pH 7.4 to the formed layer. To create small unilamellar vesicles, the liposome mixture is sonicated for a short time (Blazek 2001; Verma 2010; Tarekegn 2010; Arora 2007). A probe sonicator device is used to homogenize and sonicate the phosphotidylcholine dispersion solution for 3 min at 50% amplitude.

***Nanoparticle Characterization Test***
***Particle Size Test :***

In order to determine the size of the nanoparticles produced in the preparation, a particle size measurement test was carried out using the PSA instrument. Zeta potential. To find out the zeta potential value, it can be measured using a zeta potential analyzer. Adsorption efficiency.As many as10 ml of liposomes containing niacinamide were centrifuged at 3000 rpm for about half an hour at room temperature. To isolate the unabsorbed active ingredient, this was done. Then 5 ml of unabsorbed chemical (supernatant) was obtained, and 10 ml of distilled water was used to dilute it. Using a pipette, take 1 ml of the solution and dilute it with distilled water in a 10 ml volumetric flask. Using UV-Vis Spectrophotometry study at a wavelength of 262 nm, determine the absorbance.

$\%EE = TD - FD \,/ TD \; x100\%$.....(1)
TD: total amount of active compounds contained in the liposome formula
FD: the amount of active compound that is not absorbed.

***Anti aging activity test***

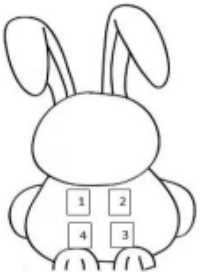
10 niacinamide liposome formulas were tested on 9 New Zealand rabbits' backs adapted for approximately one week. Shave the back fur as much as 4 parts of each rabbit with a size of 2x 2 cm in 1 part shaved carefully.

Rabbit's back part I: smeared 1 ml negative control (liposome base), 1ml positive control (anti-aging serum containing niacinamide), 1ml niacinamide liposome Run 1 and Run 2

Rabbit back part II: liposome applied 1ml niacinamide Run 3, Run 4, Run 5 and Run 6

Rabbit back part III: smeared 1ml liposome niacinamide Run 7, Run 8, Run 9 and Run 10

The test was repeated 3 times using rabbits as test animals which were treated in the same way.




Using the Skin Analyzer, the amount of collagen and elasticity of shaved back skin were determined before exposure to UV-A radiation. After that, UV-A radiation was used to induce it. Induction was done by irradiation with the Exoterra® Daylight Basking Spot device containing UV-A at a distance of 30 cm for six hours every two weeks. After induction, niacinamide liposomes were applied to the rabbits according to the treatment regimen of each group, up to 1 ml per day for 30 days. Parameters, including collagen and elasticity, were measured using the Skin Analyzer device on day 0 before liposome application (Duraivel *et al.,* 2014).

***Statistical data analysis***

Research data analysis was carried out by comparing the data obtained from the study with literature that is in accordance with the parameters. Comparing to theoretical references is done to avoid errors in the study. Nanoliposome data results were tested using the SPSSS Anova one-way test and Simplex Latex Design Software equipped with an anova test in soft were. Analysis of the selected formula was carried out by comparing the prediction data from Simplex Latex Design with the results of testing the selected formula using the t-test (T-test) with a confidence level of 95%

## RESEARCH RESULTS AND DISCUSSION

***Characterization of Nanoparticles***

The three main parameters used to characterize niacinamide liposome nanoparticles are entrapment efficiency, zeta potential, and particle size. Particle size has a significant impact on diffusion, titration, and impact processes. Particle size is often divided into three categories: The diameter of multilamellar vesicles ranges from 100 to 1000 nm. The diameter of small unilamellar vesicles ranges from 10 to 200 nm. The diameter of large unilamellar vesicles ranges from 100 nm to microns (Kozubek, 2000; Verma, 2010). Electrostatic interactions are associated with zeta potential. To avoid repulsive electrostatic interactions, the zeta potential charge must be higher than the charge of the dispersing medium. Zeta potential affects the efficacy of the drug delivery system and serves as a physical stability factor. According to Dipahayu and Kusumo (2021) the zeta potential value varies between less than -30 mV and greater than 30 mV. Liposomes that are adsorbed with an active substance are called entrapment efficiency. The percentage of active compounds that are successfully adsorbed into the carrier and can function as protection, absorption, distribution into the body, and regulated release is indicated by the entrapment efficiency value (Hudiyanti *et al*., 2022). Table 2

shows the characterization results, which are then subject to expert design analysis.

**Table 2.** Results of the Characterization Study of Niacinamide Liposome Nanoparticles

| Run | Phosphatidylcholine % | Cholesterol % | Particle Size(nm) | Zeta Potential(mV) | Adsorption Efficiency(%) |
|---|---|---|---|---|---|
| 1. | 3.3 | 1.7 | 120 | -56.89 | 97.63 |
| 2. | 1.9 | 3.1 | 354.2 | -51.99 | 96.41 |
| 3. | 0.5 | 4.5 | 424.4 | -45.11 | 96.26 |
| 4. | 4.7 | 0.3 | 101 | -69.97 | 99.00 |
| 5. | 3.65 | 1.35 | 112.9 | -60.11 | 96.87 |
| 6. | 4.7 | 0.3 | 121 | -74.19 | 97.83 |
| 7. | 2.6 | 2.4 | 137.8 | -72.2 | 96.76 |
| 8. | 4.7 | 0.3 | 119 | -76.5 | 98.02 |
| 9. | 1.55 | 3.45 | 160.9 | -53.69 | 96.24 |
| 10. | 0.5 | 4.5 | 468.7 | -48.11 | 96.78 |

Significant values of p = "prob>F", R2>0.7, Adeq Precision>4, and the difference between Adjusted R2 and Predicted R2 less than 0.2 were obtained from the design expert analysis with good model suitability for predicting the response (Indrati *et al*., 2020). These findings are consistent with the design expert analysis, as shown in Table 3, and it can be concluded that the properties of nanoparticles meet the requirements for a reliable response prediction model.

**Table 3.** Results of ANOVA Design Expert Test of Particle Size, Zeta Potential and EfficiencyAbsorption.

| Parameter | Model (p<0.05) | Lack of fit (p>0.05) | R2 | Adjusted R2 | Predicted R2 | Adeq Precision (>4) |
|---|---|---|---|---|---|---|
| Size Particle | Quadratic (0.0026) | 0.0786 | 0.8180 | 0.7660 | 0.6665 | 8.4445 |
| Zeta Potential | Linear (0.001) | 0.0885 | 0.7615 | 0.7317 | 0.6855 | 9.5335 |
| Efficiency Absorption | Linear (0.0004) | 0.7072 | 0.8054 | 0.7810 | 0.6851 | 10.8511 |

***Characterizationparticle size***

Equalityfor the particle size response is shown in equation 1.

Particle Size = 113.234 A + 423.696 B – 412.383 AB…….(1)

Note:
A = proportion of Phosphothidylcholine
B = proportion of Cholesterol.

The addition of cholesterol and phosphatidylcholine causes the particle size to increase, as shown by equation 1, which produces positive coefficients A and B. Phosphatidylcholine and cholesterol together have a negative AB coefficient, meaning that the combination will cause the particle size to decrease. The addition of cholesterol at high concentrations has a significant

effect on the increase in particle size, as can be concluded from the three coefficients produced. The value of coefficient B, which represents cholesterol, is higher than the value of coefficient A, which represents phosphorylcholine.

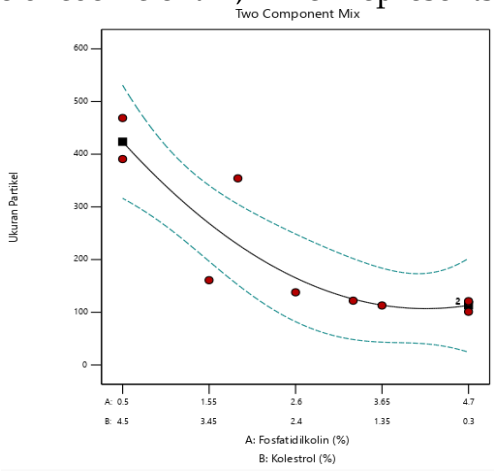



**Figure 2**. Response Curve of Particle Size with Phosphotidylcholine and Cholesterol Concentration

The particle size response curve generated by the design expert software program, it is stated that the dotted line at the bottom shows the data distribution of component B (cholesterol), and the dotted line at the top shows the data distribution of component A (phosphotidylcholine). The higher the concentration of phosphotidylcholine added, the smaller the particle size.
It can be concluded that high cholesterol concentration also results in large particle size because cholesterol dissolves in the phospholipid bilayer rather than forming a bilayer, based on the results of particle size characterization table 2, equation 1 y value generated by the design expert, and figure 1 particle response curve. The width of liposomes will increase as the cholesterol concentration increases because more cholesterol molecules will be spread throughout the phospholipid bilayer (Duangjit. S *et al*., 2014).

***Characterization of Zeta Potential***

The zeta potential response equation is shown in equation 2
ZetaPotential = -72.3068 A - 46.905B…….(2)

Note:
A = proportion of Phosphothidylcholine
B = proportion of cholesterol

The resulting equation 2 shows that the coefficients A and B are negative, indicating that the combination of cholesterol and phosphotyldylcholine causes a decrease in the zeta potential. However, it can be concluded that the presence of cholesterol in high concentrations has a significant impact on increasing the zeta potential value because the coefficient value of B (cholesterol) is greater than the coefficient value of A (phosphotyldylcholine). Ten formulas have a zeta potential value of less than -30, according to the results of the characterization study in table 2. Formulas 3 and 10 (4.5%), which contain a

significant percentage of component B (cholesterol), produce the largest zeta potentials, namely -45.11 and -48.11.

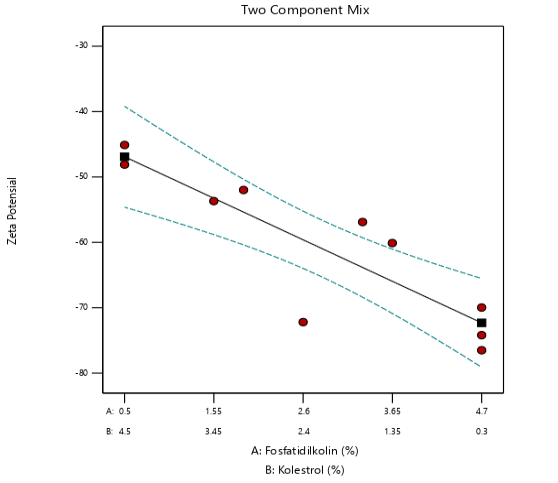



**Figure 3.** Zeta Potential Response Curve with Phosphotidylcholine and Cholesterol Concentrations

The zeta potential response curve generated by Design simplex lattex using Design Expert Software is shown in Figure 2. It is stated that the data distribution of component A (phosphotidylcholine) is shown by the upper dashed line; the addition of more phosphotidylcholine will produce a smaller zeta potential value, and the data distribution of component B (cholesterol) is shown by the lower dashed line; the higher the concentration of cholesterol used, the higher the zeta potential value. Zeta potential characterization Table 2 shows that all niacinamide liposome formulas, with zeta potential values less than -30 mV, have good stability in the nanoparticle drug delivery system (Dipahayu & Kusumo, 2021).

It can be concluded that the effect of high cholesterol concentration produces a large zeta potential value because cholesterol has a large potential for the stability of nanoparticles based on the results of the zeta potential characterization table 2, equation 2 y values produced by the design expert, and figure 2 zeta potential response curve (Tseng L, et al., 2007). Because phosphotidylcholine is positively charged and cholesterol is negatively charged, the zeta potential test produces a negative charge. Because cholesterol has a strong ability to attract positively charged electrons, cholesterol interacts with these atoms to increase its partial charge (Hudiyant, 2023).

***CharacterizationAdsorption Efficiency***

The entrapment efficiency response equation is shown in equation 3.

Entrapment Efficiency = 98.097 A + 95.86B……..(3)

Note:
A = proportion of Phosphothidylcholine
B = proportion of cholesterol

The resulting equation 3 shows that the coefficients A and B are positive, indicating that the addition of cholesterol and phosphotidylcholine increases the absorption efficiency. It can be concluded that the addition of high concentrations of phosphotidylcholine has a significant impact on the absorption efficiency because the value of coefficient A (phosphotidylcholine) is greater than the value of coefficient B (cholesterol). Ten formulations have an absorption effectiveness of more than 80%, as shown in Table 2. This finding further indicates that the highest absorption efficiency, 99.00% and 98.02%, was produced by formulas 4.8 and 6, which contain component A (phosphotidylcholine 4.7%).

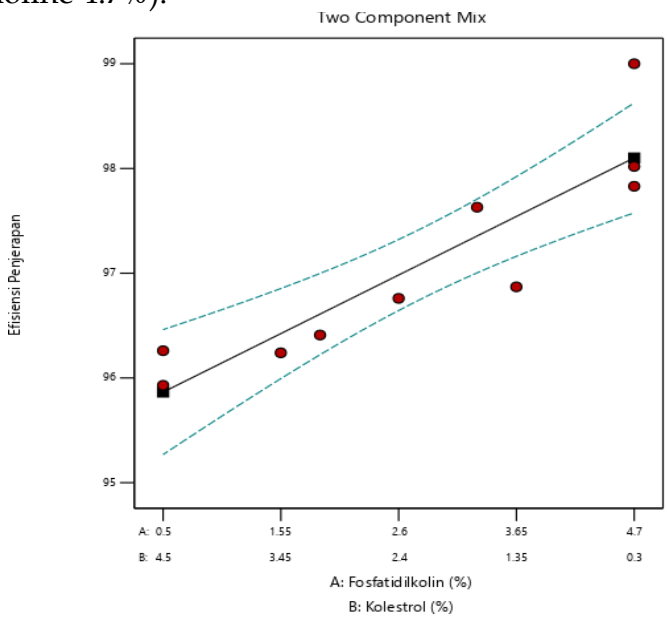



**Figure 4**. Response Curve of Adsorption Efficiency with Variation of Phosphotidylcholine and Cholesterol

The absorption efficiency response curves shown in Figure 3 were generated using the *Lattex Simplex Design* approach and the expert design software states that the data distribution of component A (phosphotidylcholine) is shown by the upper dashed line, where higher concentrations of phosphotidylcholine will result in greater absorption, and the data distribution of component B (cholesterol) is shown by the lower dashed line, where higher concentrations of cholesterol will result in less absorption.

It can be concluded that the effect of high phosphotidylcholine concentration produces a large percentage of adsorption efficiency based on the results of the adsorption efficiency characterization in table 2, the y value of equation 8 produced by the design expert, and the adsorption efficiency response curve in figure 3. This shows that the main component of the vesicles is phosphotidylcholine. The higher the concentration of phosphotidylcholine, the more vesicles are produced, so that the absorption of active compounds is likely to be more optimal (Ilham Kuncahyo, 2021).

***Anti-Aging Activity of Niacinamide Liposome Nanoparticles.***

Many cosmetics and skin care products contain niacinamide. It has been shown that niacinamide reduces skin pigmentation that causes aging (Hakozaki *et al*., 2002; Tanno *et al*., 2000). By increasing collagen synthesis, fibroblast proliferation, and elasticity, niacinamide has anti-aging effects (Salvador & Chisvert 2007; Draelos & Thaman, 2006). As a coenzyme of nicotinamide adenine dinucleotide (NAD) and NAD phosphate, niacinamide helps reduce wrinkles, red spots, and hyperpigmentation spots on aging facial skin by increasing elasticity and stimulating collagen production (Kawada *et al*., 2008). The back of the test rabbit was used to conduct in vivo anti-aging activity testing. Collagen and elasticity are anti-aging test factors that can prevent aging. Table 4 shows the findings of collagen and elasticity parameters of the anti-aging activity test. Table 5 shows the results of the expert design analysis.

**Table 4.** Results of the Anti-Aging Activity Test of Niacinamide Liposomes

| No. | Phosphatidylcholine (%) | Cholesterol (%) | Collagen (%) | Elasticity (%) |
|---|---|---|---|---|
| 1. | 3.3 | 0.7 | 28.73 | 63.55 |
| 2. | 1.9 | 3.1 | 27.37 | 62.50 |
| 3. | 0.5 | 4.5 | 24.88 | 60.87 |
| 4. | 4.7 | 0.3 | 30.79 | 66.67 |
| 5. | 3.65 | 1.35 | 28.77 | 65.06 |
| 6. | 4.7 | 0.3 | 30.30 | 66.43 |
| 7. | 2.6 | 2.4 | 27.07 | 64.29 |
| 8. | 4.7 | 0.3 | 30.49 | 66.17 |
| 9. | 1.55 | 3.45 | 26.63 | 61.70 |
| 10. | 0.5 | 4.5 | 25.56 | 60.56 |

The results of the expert design analysis of a good model balance to predict the response can be stated significantly at the value of p = "prob> F", R2> 0.7, Adeq Precision> 4, and the difference between Adjusted R2 and Predicted R2 is less than 0.2 (Indrati *et al*., 2020). The results of the ANOVA table 5 collagen response and elasticity show that it meets the criteria parameters with good model suitability to predict the response.

**Table 5.** ANOVA Design Expert Results of Anti-Aging Activity (Collagen and Elasticity)

| Parameter | Model (p<0.05) | Lack of fit (p>0.05) | R2 | Adjusted R2 | Predicted R2 | Adeq Precision (>4) |
|---|---|---|---|---|---|---|
| Collagen | Linear (0.0001) | 0.3851 | 0.9680 | 0.9640 | 0.9520 | 29,323 |
| Elasticity | Linear (0.001) | 0.0932 | 0.9625 | 0.9579 | 0.9500 | 27.0412 |

***Collagen***

Collagen Equation = 30.3968 A + 25.2016 B….....(4)

Note:

A = proportion of Phosphothidylcholine

B = proportion of cholesterol

The addition of phosphotidylcholine and cholesterol can affect the increase in collagen because according to the equation above, the values of A and B are positive. However, the value of the phosphotidylcholine coefficient (A) is higher than the value of the cholesterol coefficient (B), which indicates that phosphotidylcholine in liposomes can increase collagen. Table 5 lists ten formulas that can increase collagen. With component A (phosphotidylcholine 4.7%), formulas 4.8 and 6 showed the best results that can increase, with an increase in collagen of 30.79, 30.30, and 30.49%.

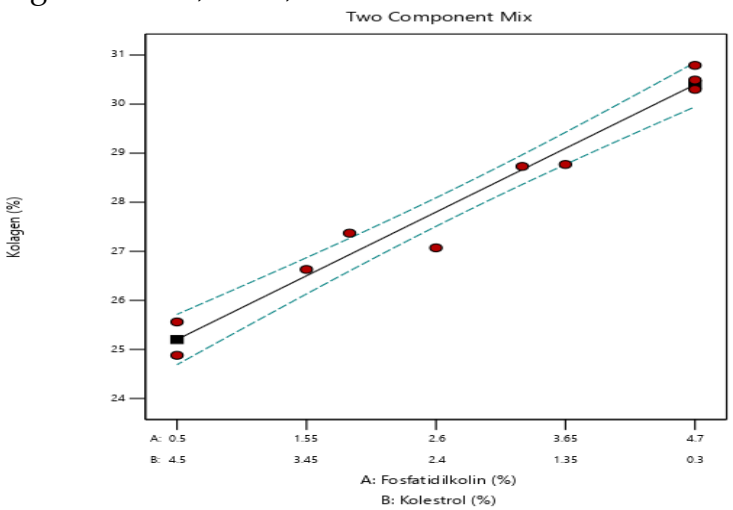



**Figure 5.** Collagen Curve with Variation of Phosphotidylcholine and Cholesterol

The resulting collagen response curve Software Design Expertusing the simplex lattex design method is shown in Figure 4. The statement is as follows: the dotted line at the bottom shows the distribution of component B (cholesterol) data; the smaller the concentration of cholesterol used, the greater the increase in the percentage of collagen. The dotted line at the top shows the distribution of component A (phosphotidylcholine) data; the addition of a larger concentration of phosphotidylcholine will result in a large increase in the percentage of collagen. Phosphatidylcholine at a concentration of 4.7% and cholesterol at a concentration of 0.3% were shown to provide a very significant increase in collagen response, according to the curve produced by the collagen response.

Nanoparticles made from phosphatidylcholine, vitamins, and melatonin have anti-aging and wrinkle-reducing properties on the skin, according to equation 4 y-values generated by Design Expert and collagen response curves in figure 4, which are based on the results of the anti-aging activity test in table 4 with collagen parameters (Morganti P., *et al*., 2012). Furthermore, it was shown that niacinamide liposomes produced a higher collagen-increasing response of 29.98% than traditional niacinamide serum (10.55%) based on observations compared to the positive control using niacinamide anti-aging serum. Solid lipid nanoparticles and niacinamide liposome drug delivery technology can both enhance penetration to optimize its effects.

***Elasticity***

Elasticity equation = 66.3557 A + 60.632 B……(5)

The addition of cholesterol and phosphotidylcholine can affect the increase in elasticity, as shown by the equation above, where the values of A and B are positive. The fact that the value of coefficient A (phosphotidylcholine) is higher than the value of coefficient B (cholesterol) indicates that the concentration of phosphotidylcholine in liposomes can further increase elasticity. Table 5 also shows these results. The addition of 4.3% phosphotidylcholine to formulas 4, 6, and 8 resulted in an increase in elasticity of 66.67, 66.43, and 66.17%.

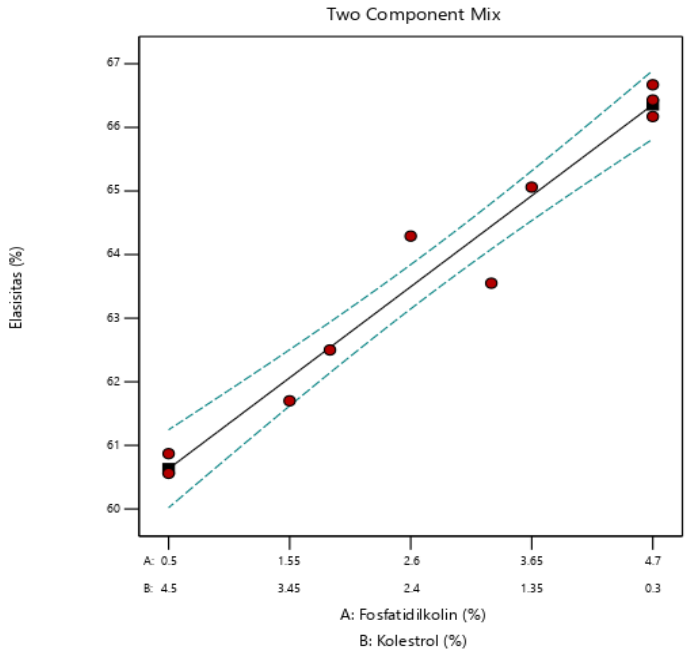



**Figure 6.** Elasticity Response Curve with Variation of Phosphotidylcholine and Cholesterol

The elasticity response curve generated by the simplex lattex design approach using the software design expert is shown in Figure 5. It is stated that the distribution of component A data, phosphotidylcholine, is shown by the upper dashed line. The addition of more phosphotidylcholine will cause a significant increase in the percentage of elasticity. The distribution of component B data, cholesterol, is shown by the lower dashed line. The greater the increase in the percentage of collagen, the smaller the concentration of cholesterol used. Phosphatidylcholine at a concentration of 4.7% and cholesterol at a concentration of 0.3% were shown to provide a very significant increase in the elasticity response, according to the curve generated by the elasticity response.

Nanoparticles containing phosphatidylcholine, vitamins, and melatonin have wrinkle-reducing and anti-aging effects on the skin, according to the results of the anti-aging activity test table 5 with elasticity parameters, equation 10 y values generated by Design Expert, and elasticity response curve figure 15 (Morganti P., *et al*., 2012). Furthermore, it was found that niacinamide liposomes produced an increased elasticity response of 65.56% greater than

standard niacinamide serum, which was 45.10%, based on observations compared to the positive control using conventional niacinamide anti-aging serum. Solid lipid nanoparticles and niacinamide liposome drug delivery technology can both increase permeability to produce faster effects (Renata Basto, 2021).

***Determination of Optimum Nano Particle Formula***

The data from the liposome characterization test and anti-aging test were used to optimize the formula using the Design Expert 13 software.

**Table 6.** Optimal Formula Criteria Parameters

| Name | Goal | Lower Limit | Upper Limit | Lower Weight | Upper Weight | Importance |
|---|---|---|---|---|---|---|
| A:Phosphatidylcholine | Is in range | 0.5 | 4.7 | 1 | 1 | 3 |
| B: Cholesterol | Is in range | 0.3 | 4.5 | 1 | 1 | 3 |
| Particle Size | Is in range | 100 | 200 | 1 | 1 | 3 |
| Zeta Potential | Minimize | -76.5 | -30 | 1 | 1 | 3 |
| Adsorption Efficiency | Maximize | 81 | 100 | 1 | 1 | 3 |
| Collagen | Maximize | 24.88 | 30.79 | 1 | 1 | 3 |
| Elasticity | Maximize | 60.56 | 66.67 | 1 | 1 | 3 |

**Table 7.** Optimal Formula Results

| No | Phosphatidylcholine | Cholesterol | Particle Size | Zeta Potential | Adsorption Efficiency | Collagen | Elasticity | Desirability |
|---|---|---|---|---|---|---|---|---|
| 1 | 4,700 | 0.300 | 113,528 | -65,9564 | 97.5423 | 29,098 | 64,9247 | 0.923 Selected |

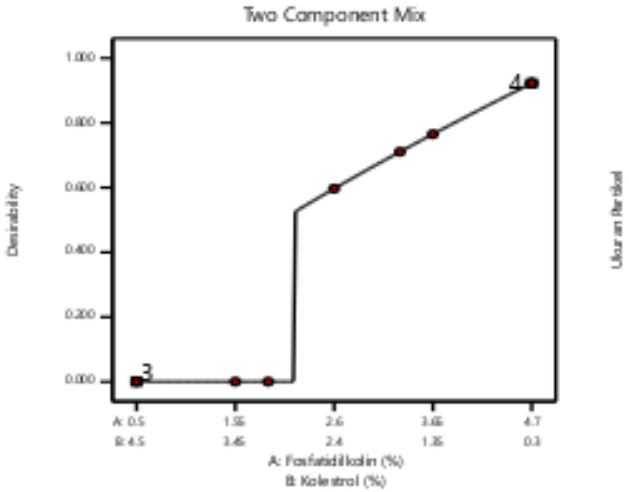



**Figure 7.** Optimal Formula Curve

Table 7 shows that the ideal formula results are produced with a phosphotidylcholine factor of 4.3% and cholesterol of 0.3% with a desirability value of 0.923, based on the results of the criterion parameter analysis in Table 6. These results are in accordance with Figure 6 of the optimal formula curve which states that the optimal formula consists of a phosphotidylcholine A coefficient of 4.3% and a cholesterol B coefficient of 0.3% and shows the largest desirability value. The desirability value approaching 1 indicates that the actual response value is likely not significantly different from the predicted results. The expected particle size value was also obtained at 113.528, zeta potential -65.9564, from the optimal formula results in Table 8.

**Table 8.** Optimal Formula Test Results

| Analysis | Predicted Mean | Predicted Median | Observer | Std Dev | N | SE Pred | 95% Pl Low | Mean Data | 95% Pl High |
|---|---|---|---|---|---|---|---|---|---|
| Particle Size | 113,528 | 113,528 | | 67,1237 | 1 | 73,4061 | -60,0499 | 135.7 | 287,106 |
| Zeta Potential | -65,1307 | -65,1307 | | 5.95798 | 1 | 6,32909 | -80,5513 | -77.19 | -51,3614 |
| Adsorption Efficiency | 83.4523 | 83.4523 | | 0.460843 | 1 | 0.489549 | 82,4134 | 98.01 | 98.6712 |
| Collagen | 25,5712 | 25,5712 | | 0.396161 | 1 | 0.420837 | 24,1276 | 29.98 | 30,0685 |
| Elasticity | 58,0845 | 58,0845 | | 0.473301 | 1 | 0.502782 | 56,7653 | 65.56 | 66,0842 |

Using the IBM® SPSS® Statistics 25 program to compare the test result responses with the estimated optimal formula responses obtained from the analysis using Design Expert Software version 13. Since all data are regularly distributed, the one-sample t-test statistical analysis approach was used.

**Table 9.** Results of One Sample T-Test

| Parameter | Prediction | Testing | Sig | Interpretation |
|---|---|---|---|---|
| Particle size | 113,528 | 135.7 | 0.56 | No significant difference |
| Zeta potential | -65,1307 | -77.19 | 0.54 | No significant difference |
| Adsorption efficiency | 83.4523 | 98.01 | 0.51 | No significant difference |
| Collagen | 25,5712 | 29,9811 | 0.5 | No significant difference |
| Elasticity | 58.0845 | 65.56 | 0.5 | No significant difference |

Comparing the test result response with the estimated ideal formula response obtained from the analysis using Design Expert Software version 13 using the IBM® SPSS® Statistic 25 program. The statistical analysis method used is the one-sample t-test because all data is normally distributed.

## CONCLUSIONS AND RECOMMENDATIONS

1. By adding high cholesterol concentrations, particle size can be increased and zeta potential can be increased.
2. The optimum formula was produced by adding phosphotidylcholine with a concentration of 4.7% and cholesterol 0.3% resulting in a large particle size of 135.7 nm, zeta potential - 77.19 mV, and adsorption efficiency of 98.01%.
3. The liposome formulation of niacinamide has anti-aging activity which was tested on the backs of New Zealand rabbits exposed to UV-A light which showed an increase in collagen value of 29.98% and elasticity of 65.56%.
4. Niacinamide preparations provide a significant increase in anti-aging activity andincrease permeability so that it can provide a faster effectcompared to niacinamide serum without liposomes.

## ADVANCED RESEARCH

1. Further researchers can make liposome nanoparticles with other methods to obtain smaller particles**.**
2. Further researchers are expected to be able to make pharmaceutical preparations of niciamide liposome nanoparticles
3. Further researchers are expected to be able to conduct other pharmacological activity tests
4. Further researchers are expected to be able to conduct morphological tests of niciamide anti-aging liposome nanoparticles

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