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Original Research Article

Optimization of Formula Using D-Optimal Design Method and Stability Testing of Ascorbic Acid Transferosomes Gel

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ABSTRACT

Ascorbic acid is an active pharmaceutical ingredient in cosmetics with antioxidant activity and is used in dermatological practice as a preventive measure against photo-ageing and hyperpigmentation. The development of transferosomes in topical gel preparations may facilitate its application and have potential as nano-cosmeceuticals. This study aims to develop ascorbic acid transferosome formulations using the vortex sonification method to meet the characterization requirements to obtain a stable ascorbic acid transferosome gel preparation based on the optimum formula from the optimization results of Carbopol 940 and propylene glycol using the D-optimal design method. The results showed the characterization of ascorbic acid transferosomes with a particle size of 152.2 nm, a poly-dispersity index value of 0.571, and a zeta potential of -36.50 mV. The optimum formulation produced a Carbopol 940 concentration of 0.8% and a propylene glycol concentration of 3.7% with a desirability value of 1. The stability results with the cycling test method produced a relatively stable gel preparation.

Keywords: Transferosomes, Ascorbic Acid, Gel, D-Optimal Design, Carbopol, Propylene glycol

Introduction

Cosmetics are dosage forms that have long been used by humans and include a wide variety of products that are applied to the outer surface of the human body to cleanse, perfume, change the appearance, remove odours emitted by them, or keep the area of the body to which they are applied in generally good condition.[1] There is also the term cosmeceutical, which refers to a cosmetic product containing therapeutic active ingredients with a curative effect and a therapeutic purpose in its application.[2] The role of cosmetics and cosmeceuticals is growing, with an increasing demand for innovative products based on scientific knowledge and technological development.[1] The introduction of nanotechnology has great potential to generate innovations in formulations by overcoming the drawbacks associated with conventional cosmetics or cosmeceuticals and has provided improved properties in their formulations. Formulating cosmetics and cosmeceuticals with nanotechnology is a relatively new field called nano cosmeceuticals.[2]

Cosmetic preparations consist of active substances and other excipients to form the base and carrier of the preparation.[1] Ascorbic acid is one of the active substances that can be used in the manufacture of cosmetics, and it can be applied topically in dermatology as a preventive measure related to photo ageing and hyperpigmentation. However, administering ascorbic acid to the skin through topical preparations has always been challenging.[3] The biggest challenge in ascorbic acid is maintaining stability and enhancing its delivery to the receptor's active site[4] due to its poor skin penetration because of the hydrophobic nature of the stratum corneum, and ascorbic acid tends to be unstable.[3]

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One strategy that has been and is being developed is the development of drug carrier or vesicle delivery systems.[4] A drug delivery system in the formulation of nanoparticles enhances therapeutic value by declining toxicity, extending bioavailability, and advancing drug stability against enzymatic degradation.[5] One of them is transferosomes, which are ultra-deformable vesicles consisting of phospholipids, edge activators, and organic solvents with a hydrated core part and surrounded by a complex lipid layer that makes transferosomes have self-optimising and self-regulating capabilities.[7,3] The ultra-deformable vesicle system is highly elastic and flexible to penetrate the deepest layers of the skin in one piece.[8] When the transferosomes reach the skin pores, the system can change its membrane flexibility and pass through them spontaneously, called self-optimising.[6] Another advantage of ascorbic acid with transferosomes as a carrier system is its relatively high entrapment efficiency. Almost as much as 90% when it comes to hydrophilic drugs.[7].

Gel preparations are of great interest to the drug and cosmetic industry as they are effective and safe options for treating and managing skin-related diseases with minimised side effects compared to conventional preparations. Topical gel preparations also provide a more comfortable impression when applied, have good drug release, and have better stability when compared to creams and ointments.[9] Gel formulation has several essential components, including gelling agents and humectants. The gelling agent used in this study is Carbopol 940 because it is non-toxic and non-irritating with repeated use, easily dispersed in water, even water at room temperature (without heating), and will produce transparent/precise gel preparations. In addition, Carbopol 940 was selected because it has an extensive viscosity range, which allows it to form a gel base with a minor concentration.[10] However, a humectant is needed to improve the properties of Carbopol 940 as a gelling agent and maintain the stability of the gel.[11] The humectant used in this study is propylene glycol due to its wide use in cosmetic preparations.[12] Propylene glycol can bind with water and form hydrogen bonds to prevent water's evaporation from the gel preparation, which can affect the physical stability of the gel.[13] The concentration of Carbopol 940 as a gelling agent and propylene glycol as a humectant must be considered to produce an excellent and stable gel preparation. Therefore, it is necessary to optimise gel preparations for an optimum formula and high stability. The optimisation method used is the D-optimal mixture design method using Design Expert software version 14. One of the advantages

of this method is that it is practical and fast because formula determination is not done by trial and error.[14] In addition, the D-optimal design method tends to produce fewer runs, so it is cost-efficient.[15] The preparation stability test was also carried out using the cycling test method to ensure that the ascorbic acid transferosome gel preparation quality met the expected specifications and was stable during storage based on the optimal formula.

This study formulated ascorbic acid transferosome gel using lecithin as a vesicle former, tween 80 as a vesicle elasticity enhancer, and ethanol as an organic solvent and edge activator. The reason for developing ascorbic acid transferosomes into a topical gel preparation in this study is because it is easier to apply to the skin, and ascorbic acid transferosomes have the potential as nano cosmeceuticals in dermatology practice. This is the first report of ascorbic acid transferosome gel formulation as a nanocosmeceutical from the literature search.

Materials and Methods

Materials

The ingredients used were Ascorbic acid 99.7% (Merck®, Germany), Lecithin (Shankar Soya Products®, India), Tween 80 (Merck®, Germany), Ethanol pro analysis (Merck®, Germany). Carbopol 940 (Lubrizol® USA), Propylene glycol USP (Brataco®, Indonesia), Triethanolamine (Merck®, Germany), Benzalkonium chloride (Kao®, Indonesia), distilled water.

Methods

Formulation of Ascorbic Acid Transferosomes (AAF)

Ascorbic acid Transferosomes were prepared using the vortex-sonication method. Transferosomes were made by mixing 750 mg of lecithin, 250 mg of tween 80, and 14.29 mL of ethanol (Table 1) with vigorous shaking or stirring using a vortex to form a suspension mixture.[16] About 100 mg of ascorbic acid is added to the suspension mixture. Furthermore, constant stirring was carried out using a magnetic stirrer. Sonication was carried out using a bath sonicator at 60ºC for 30 minutes to reduce the particle size.[17]

Characterization of AAF Particle Size

The particle size of AAF was determined using a Particle Size Analyser (PSA) with the Dynamic Light Scattering (DLS) method. One drop of each preparation transfer was dispersed into 10 mL of distilled water in a cuvette and tested for particle size using a Particle Size Analyser (PSA) at 25ºC. The particle size of the transfer of some globules will be measured.[18]

Polydispersity Index (PDI) value

The polydispersity index value is the same as the determination of particle size, which can be accomplished using the Particle Size Analyzer (PSA) tool with the principle of the Dynamic Light Scattering (DLS) method. One drop of each Transferosomes preparation was dispersed into 10 mL of distilled water in a cuvette and tested for the polydispersity index (PDI) value using a Particle Size Analyser (PSA) at 25ºC. The polydispersity index (PDI) value will be measured.[18]

Zeta potential

Characterization was performed by measuring the surface charge of nanovesicles in capillary cells using a Zeta Potential Analyser (Zetasizer) or Particle Size Analyzer (PSA). Measurement of zeta potential can predict stability.[19] Particles with a zeta potential value ± 30 mV are considered stable and good.[18]

Ascorbic Acis Transferosome Gel Formula Optimisation

Determining variations in the concentration of Carbopol 940 and propylene glycol was optimised using the D-optimal design method in Design Expert software version 13. Two factors were the independent variables, namely Carbopol 940 and propylene glycol, and the response was the dependent variable, namely pH, viscosity, and spreadability. The analysis results of this method resulted in 8 runs, which are presented in Table 2. The gel formulation is illustrated in Table 3.

Table 1: The formula of Ascorbic acid transferosomes (Ismail et al., 2018)

Material Name	Material Weight
Lecithin	750 mg
Tween 80	250 mg
Ethanol	14.29 ml
Ascorbic acid	100 mg

Table 2: Concentration of Carbopol 940 and Propylene Glycol

Run	Carbopol 940 (%)	Propylene Glycol (%)
1	0.88	3.62
2	1.62	2.88
3	2	2.5
4	1.25	3.25
5	0.5	4
6	2	2,5
7	1,25	3,25
8	0,5	4

Note: *Run* = nominal experimental

Table 3: Ascorbic Acid Transferosome Gel Formulas

Material Names	Function	Formula (% b/b)				
		F1	F2	F3	F4	F5
Transferosomes	Active Ingredients	0.10	0.10	0.10	0.10	0.10
Carbopol 940	Gelling agent	0.50	0.88	1.25	1.62	2.00
Propylene glycol	Humectant	4.00	3.62	3.25	2.88	2.50
Triethanolamine	Alkalising agent	0.50	0.50	0.50	0.50	0.50
Benzalkonium chloride	Preservative	0.02	0.02	0.02	0.02	0.02
Aqua destilata	Solvent	ad 100	ad 100	ad 100	ad 100	ad 100

Ascorbic Acid Transfersom Gel Preparation ((AATG)
AATG was prepared using the dispersion method, which was carried out by mixing the gelling agent with water until the gelling agent expanded. Then, the drug was dissolved in the solvent medium and incorporated into the gel base.[9] The gel formula has varying concentrations of Carbopol 940 as a gelling agent, dissolved in 20 mL of distilled water at 70ºC in a glass beaker. All excipients (triethanolamine, benzalkonium chloride, propylene glycol) were added to each formula and mixed until homogeneous. Ascorbic acid was added to the solvent media mixture and stirred homogeneously using a magnetic stirrer to become a mixture.[20]

Evaluation of AATG
Organoleptic Test
The organoleptic test was performed by observing the gel preparation's appearance.[21] The Colour, flavour, texture, and odour were evaluated.[22]

Homogeneity Test
Homogeneity testing was performed by placing 0.5 g of gel preparation on a glass object and then covering it with another. A good gel preparation is homogeneous, indicated by the absence of powdered granules of gelling material.[11]

pH test
The pH test was conducted using a pH meter calibrated with pH 4.01, pH 7, and pH 10.01 buffer solutions. The pH meter electrode was sipped into 10 mL of gel preparation solution that had been dissolved as much as 1 g, and the pH value was read. The gel should have a pH in the normal skin pH range of 4.5-6.5.[23]

Viscosity Test
A viscosity test was conducted using a Brookfield viscometer. 100 g of gel was placed in a glass beaker, and spindle no.6 was used at 50 rpm for 60 seconds.[24] Furthermore, the viscometer was run to show the magnitude of the viscosity based on SNI 16-4399-1996, which is 2000-50,000 cPs.[25]

Spreadability Test
The formulated gel (1 g) was placed between two glass plates, and then a 125 g weight was put on it for 1 minute. The gel's diameters were measured using a calliper.[26] The requirement for good gel spreadability is 5–7 cm.[27]

Stickiness Test
About 0.5 g of gel preparation was placed on the object glass and then covered with another object glass until it merged. A load weighing 1 kg was placed on both object glasses for 5 minutes. After completion, the load was taken, and 80 g were loaded. A release load of 80 g, starting the stopwatch and recording the time it took for the two object glasses to be released, was recorded.[28]

Syneresis Test
The syneresis test was carried out by storing the gel at 5°C and 35°C for 72 hours, with observations made at the 24th, 48th, and 72nd hours. The gel was placed in a container or cup to see the release of water during the storage time.[29] The resulting syneresis percentage value must be <5%.[30]

Data Analysis
Response Analysis
The responses generated from the experimental results of ascorbic acid transferosome gel preparations, including pH, viscosity, and spreadability, were analysed using the ANOVA (Analysis of Variance) statistical test in the Design Expert version 13 program. The ANOVA test helps determine the significance of response analysis between variables. It can determine the model suggested by the Design Expert, characterized by a proposed model.[14] 95% confidence was used in all of the analyses performed for this study.[5]

Optimum Formula Analysis
Optimization was done by determining the limits of the desired response criteria with a range that can be achieved. The most optimum formula is the formula with the maximum desirability value, which is closer to 1.0. The desirability value is a function value in the optimization objective that shows the program's ability to fulfil desires based on the criteria set for the final product so that it shows the program's ability to produce the desired product more perfectly.[14]

Confirmation of Optimum Formula
Confirmation or validation experiments of the optimum formula can be done using the relative error value between the response value predicted and the response's actual measurement (experiment) value by the Design Expert. The relative error results must be less than 5% (α = 0.05). The Relative Error (RE) percentage between the predicted and actual measurement of responses was calculated using Equation 1.

$$RE\ (\%) = [Predicted/\ Real\ Experiment] \times 100 \quad (1)$$

Stability Cycling Test Method
The physical stability test of the gel was carried out using the cycling test method. The stability test was conducted by storing the gel preparation containing the optimum formula for six cycles (12 days). One storage cycle consists of 48 hours of storage at 4±2°C (cold temperature) for 24 hours, then transferred to an oven at 40±2°C (one cycle).[32]

Results and Discussion

Transferosomes containing ascorbic acid were formulated at 100 mg/g for the skin delivery system (Figure 1). The organic solvent used in the formula was 7% b/v ethanol. The composition of ethanol content in vesicles can affect the deformability of the transferosome membrane, affecting its penetration ability. The basis for choosing ethanol as an organic solvent is the solubility of the active substance ascorbic acid, which dissolves in 30 to 100 parts of ethanol.[21] Ethanol is also a safer organic solvent than others, such as methanol and chloroform.33 Lecithin and edge activators were used in a ratio of 75:25, respectively, to determine the flexibility of the Transferosomes.19 Transferosomes have good colour intensity and a yellowish solution (light yellow). They are opalescent/cloudy and smell typical of soy lecithin. The yellow colour of transferases is obtained from the colour of the soy lecithin used. The more soy lecithin is used, the more intense the colour intensity. In addition, the 80 used in this study also produce good physical properties of the transferosomes.[17] The particle size of ascorbic acid Transferosomes presented in Table 4 shows a result of 152.2 nm. This can be influenced by the appropriate ratio of phosphatidylcholine and edge activator, which is 75:25, respectively. Increasing the surfactant concentration (Tween 80) in transferosome vesicle formation while staying within the limit will result in a smaller particle size. Increasing the amount of phosphatidylcholine will result in a larger particle size.[1] This is because the surfactant in the formulation will adsorb at the interface or absorb into the lipid layer to reduce the interfacial tension of each phase, leading to particle size reduction.
Measurement of the polydispersity index value determines the physical stability of a dispersion system, where a low polydispersity index value indicates that the dispersion system formed is more homogeneous and stable for the long term.18 The resulting polydispersity index value based on Table 4 is 0.571. PDI values above 0.7 indicate that the sample has an extensive particle size distribution and is probably unsuitable for analysis by the dynamic light scattering (DLS) technique. Also, the resulting zeta potential values based on Table 4 were -36.50 mV, indicating colloidal stability.[34] Charges above +30 mV and below ~30 mV have significant cationic and anionic properties, respectively, suggesting that they can resist one another and result in minimal particle

aggregation.[35] The negative zeta potential value is due to the formula's surfactant components (lecithin). The presence of lecithin, one of the zwitterionic surfactants that are stable over a wide pH range (pH 2-8), shows a high negative charge exceeding -30 mV. The higher the pH value reaches 8, the more negative it is because it decreases the concentration of H+. In addition, the reduced negative charge can be related to the protonation of the phosphate group of the lecithin molecule.[34] The colour produced in gel preparations with low concentrations is clear/transparent. However, the colour tends to change to slightly foggy or cloudy as the concentration of Carbopol 940 increases (Figure 2). The same thing also happens to the consistency of the resulting viscosity, with an increase in the concentration of Carbopol 940 and a decrease in the concentration of propylene glycol in all formulas. Formula 4 and Formula 5 have a slightly thicker consistency. This is because the increase in Carbopol 940 concentration increases reactive sites for the polymerisation reaction. The higher the concentration of Carbopol, the higher the reactive sites available for monomer polymerisation and the more significant the gel fraction.[35] In addition, the gel will be thicker with increased viscosity and decreased spreadability.

Table 4: Characterisation results of 100 mg/g ascorbic acid transferosomes

Particle Size Diameter	Polydispersity Index (nm)	Zeta Potential (mV)
152.2	0.571	-36.50

Figure 1: Ascorbic acid transferosomes

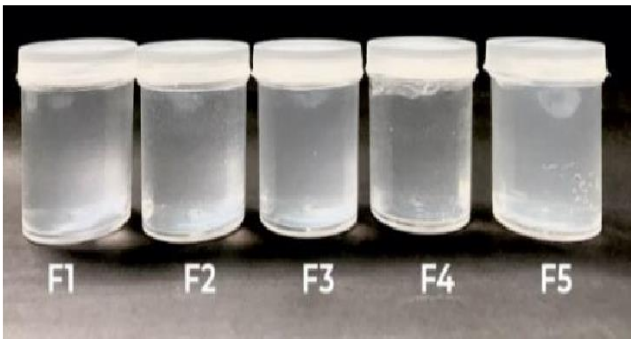


Figure 2: Ascorbic acid transferosomes gel

The test results of the evaluation of the five ascorbic acids transferosome gels entered into ANOVA analysis to the response in Design Expert software version 13 with the D-Optimal Mixture Design method are presented in Table 5. In this study, the independent variables or component factors consisted of Carbopol 940 (A) and propylene glycol (B), and the dependent variables or responses consisted of pH, viscosity, and spreadability. The ANOVA analysis was carried out using Design Expert software version 13. The parameters of good ANOVA results are p-value < 0.05, R2 result close to 1, adequate precision > 4, and lack of fit model p-value > 0.05 (insignificant). A negligible lack of fit value is required for a good model because it indicates the fit of the response data with the model.[36] All three responses have met the parameters of a suitable ANOVA. The R2 value that is less than 1 indicates that at least some of the variables in the data cannot be explained by the model. For the result, R2 of 0.5 suggests that 50% of the variability in the outcome data cannot be explained by the model, which is the error component in the model (Table 6). The determinants of regression analysis are the relationship between a dependent and independent variable and the statistical hypothesis that all other variables stay settled. The measure of the connection outcomes in a theoretical straight line and the correlation coefficient (r) calculates how near the experimental data are to the theoretical straight line we have computed. One of the problems is that variations in the population under study can significantly affect the magnitude of R2. Therefore, there is no guarantee that a high coefficient of determination indicates how well the line fits the data. Similarly, there is no guarantee that a negligible R2 suggests a weak relationship, given that variations largely influence the statistics in the independent variables. The analysis should include information from all available statistical parameters, so no benchmark value is used explicitly for R2. [37]

Table 5: Response results of the five ascorbic acid transfersom

Run	Formula	pH	Viscosity	Spreadability
5	F1	6.75 ± 0.01	14066.67 ± 705.50	5.49 ± 0.09
8	F1	6.82 ± 0.09	14526.67 ± 110.15	5.64 ± 0.10
1	F2	6.18 ± 0.34	14913.33 ± 845.06	5.24 ± 0.06
4	F3	5.49 ± 0.10	13926.67 ± 233.52	5.22 ± 0.02
7	F3	5.44 ± 0.05	14640 ± 831.62	5.19 ± 0.06
2	F4	5.28 ± 0.11	15146.67 ± 422.53	5.05 ± 0.01
3	F5	4.73 ± 0.04	15380 ± 321.87	4.96 ± 0.03
6	F5	4.75 ± 0.02	16680 ± 288.44	4.86 ± 0.19

Table 6. ANOVA analysis and response *fit statistic*

Parameter Analysis	pH	Viscosity	Spreadability
Suggested Model	Quartic	Linear	Linear
Model (p-value <0,05)	<0,0001	0,0385	0,0001
Lack of Fit (p-value >0,05)	-	0,4886	0,4458
R^2	0,9992	0,5375	0,9232
Adeq Precision (>4)	71,7433	4,9794	16,0089

ANOVA analysis produces coefficients of various factors on the response, then made as an equation formula. The polynomial equation is used to conclude after considering the magnitude of the variable coefficient on the response and the mathematical sign it carries, which is positive or negative. A positive sign signifies synergy, representing an expected change affecting the results (Table 7). The coefficient value of the variable in the equation shows how much influence the factor has on the measured response compared to other factors. A positive sign in front of the coefficient value of A, B, or AB in the equation indicates that as the value of the factor increases, the response results will increase and vice versa.[38] The model graph of the relationship of component factors with response is (a) pH, (b) viscosity, and (c) spreadability. Table 8 shows the upper and lower limits of the independent and dependent variables with a numerical approach to determine the desired target criteria in obtaining the optimum formula for ascorbic acid transferosome gel preparation in the Design Expert software version 13 D-Optimal Mixture Design method. The optimization results in the form of factor concentration solutions and optimum response predictions obtained from Design Expert software version 13 D-Optimal Mixture Design method with the results of desirability value are presented in Table 9. Therefore, the optimum formula for ascorbic acid transferosome gel is shown in Table 10.

The results of evaluating the optimum formula of ascorbic acid transfer gel, including organoleptic testing, homogeneity, pH, viscosity, spreadability, and stickiness, are presented in Table 11. The presence of air bubbles can be caused by several factors, including the process and speed of stirring and adding triethanolamine immediately into the gel base (Figure 4). When neutralised by triethanolamine, the gel base will bind air, and trapped air can form bubbles. Moreover, Carbopol has a reasonably high ability to capture air. However, air bubbles in the preparation will decrease depending on the length of time and storage temperature and the relation between the work done by buoyancy and the whole yield stress, viscous scattering, and surface tension.[38]

Table 7: Equation results for each response

Response	**Equation**
pH	$Y = 4,74*A + 6,79*B – 1,19*A*B + 0,6533*A*B*(A-B) + 4,07*A*B*(A-B)^2$
Viscosity	Y = 15706,11 A + 14113,89 B
Spreadability	Y = 4,89 A + 5,52 B

Table 8: Variables and constraints to determine the optimum formula

Independent Variable	**Low Composition**	**High Composition**	***Goal***
A: Carbopol 940	0.5%	2%	*In range*
B: Propylene Glycol	2.5%	4%	*In range*
Dependent Variable	Lower Limit	Upper Limit	*Goal*
pH	4.5	6.5	*In range*
Viscosity	2000 cP	50.000 cP	*In range*
Spreadability	5 cm	7 cm	*In range*

Table 9: Recommendations for optimum concentrations of factors and responses

Carbopol 940 (%)	**Propylene Glycol (%)**	**pH**	**Viscosity (cP)**	**Spreadability (cm)**	***Desirability***
0.800	3.700	6.357	14432.333	5.394	1.000

Table 10: The optimum formula of ascorbic acid transfersom gel

Material Name	Usability	**%b/b**
Transfersom Ascorbic acid	Active substance	0.1
Carbopol 940	*Gelling agent*	0.8
Propylene glycol	Humectants	3.7
Triethanolamine	*Alkalising agent*	0.5
Benzalkonium chloride	Preservatives	0.02
Aqua distillate	Solvent	ad 100

Table 11: Evaluation results of the optinum formula of ascorbic acid transfersom gel

Evaluation	**Results**
Organoleptic	Clear color, characteristic odor, slightly runny gel, very few air bubbles
Homogeneity	Homogeneous (no lumps of particles)
pH ± SD	6,31 ± 0,01
Viscosity ± SD (cP)	14506,67 ± 272,27
Spreadability ± SD (cm)	5,4 ± 0,32
Adhesion ± SD (detik)	4,59 ± 0,32

Table 12: Syneresis test results of the optimum formula of ascorbic acid transfersom gel

Temperature	24 hour (%)	48 hour (%)	72 hour (%)
Five ℃	0.04	0.04	0.06
35℃	0.16	0.08	0.44

In addition, the results of the syneresis test of the optimum formula of ascorbic acid transferosome gel at 5ºC and 35ºC for 72 hours are presented in Table 12. This weight loss is due to decarboxylation, the development of unsaturated structures, and depolymerisation of the polymer. The rate of syneresis increases with increasing temperature, as syneresis involves kinetic phenomena. The closed porous liquid must move through the solid structure to obtain a shrinkage that depends on the viscosity of the absorbent liquid and the polymerisation reaction rate.[40] The observation of syneresis during storage showed no significant weight loss and was relatively stable (Table 12). This is because there is a greater concentration of propylene glycol than Carbopol 940 in the optimized formula, where propylene glycol can bind with water and form hydrogen bonds so that it will prevent evaporation of water from the gel preparation so that the results of syneresis in this study show promising results that meet the requirements of <5%.[29] The results of making the optimum gel formula were evaluated (Tables 13,14 and 15), and three times of data acquisitions were carried out so that the average value of the response results was obtained namely pH 6.313±0.01, viscosity 14506.7±272.27, and spreadability 5.4±0.32. All actual results of the optimum formula response testing are within the predicted interval values are pH = 6.357 (6.209– 6.505), viscosity = 14432.3 (13284.2– 15580.5), and spreadability = 5.394 (5.253– 5.534). The actual level (α) used is 5% (α = 0.05), the confidence level is 95%, the tolerance proportion is 0.99 (99% population), and the upper and lower value intervals are two-sided. This means that approximately 5 out of every 100 conclusions will reject the hypothesis that should be accepted, and about 95% believe that the conclusion made is correct. Therefore, the hypothesis has been dismissed at a fundamental level (α) of 0.05, which means the probability of being wrong with a chance of 0.05.[31] The response results are entered into the confirmation table in the post-analysis section. This confirmation stage is intended to validate or ensure that the model can predict actual results almost by the optimal settings determined from the analysis. This confirmation stage is done by comparing the prediction interval value of the model with the average actual value. The model is confirmed if the average value is within the prediction interval. In addition, the relative percentage error values obtained vary from 0.12% to 0.68%, which is <5%, so it can be verified that the experimental/actual data is by the predicted data. In this study, it can be said that the model is confirmed and verified because all the optimum formula response results are within the prediction interval and have a relative percentage error value <5%.[31]

Table 13: Experimental test results on the optimum formula response

Data	pH	Viscosity	Spreadability
I	6.33	14200	5.60
II	6.31	14600	5.57
III	6.30	14720	5.03

Table 14: Confirmation results of the optimum formula

Response	Predicted Mean	95% PI low	Data Mean (Mean ± SD)	95% PI high
pH	6.35712	6.20951	6.31 ± 0.01	6.50473
Viscosity	14432.3	13284.2	14506.7 ± 272.27	15580.5
Spreadability	5.39358	5.25353	5.4 ± 0.32	5.53364

Table 15: Organoleptic test results of the optimum formula for six cycles

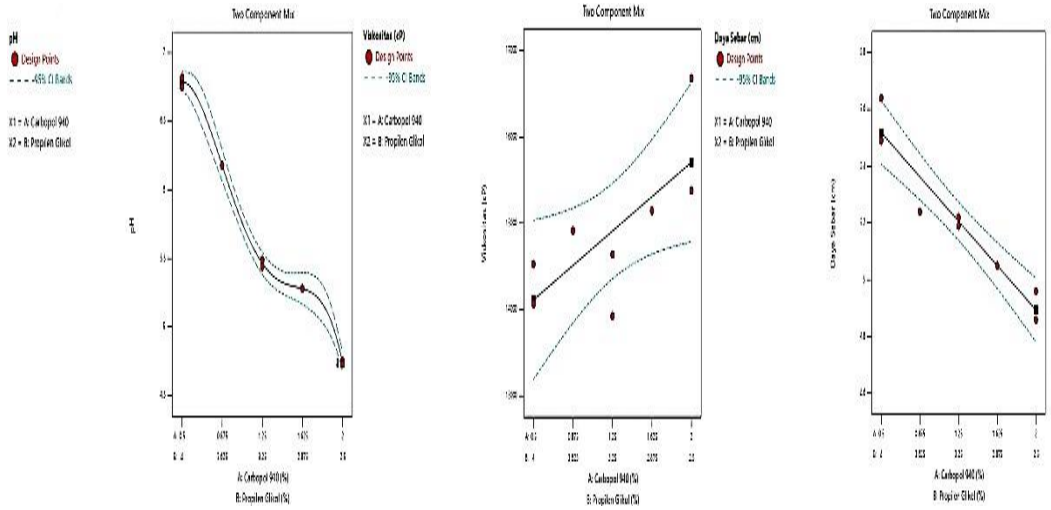
Cycle	Color	Smell	Shape	Textur (consistency)	Air Bubbles
0	Clear	Typical	Gel	Somewhat watery	Very Little
1	-	-	-	-	-
2	-	-	-	-	+
3	-	-	-	-	+
4	-	-	-	-	+
5	-	-	-	-	+
6	-	-	-	-	+

Description:
(-) = no change
(+) = there is a change in the formula

Stability testing of the optimum formula was carried out using the cycling test method at 4ºC and 40ºC for six cycles. Observations were made by looking at the results of changes in the organoleptic, pH, viscosity, spreadability, and Stickiness tests. The temperature cycle test is an accelerated physical method commonly used in pharmaceutical science to provide information about the instability of a product that isothermal testing does not provide. The cyclic temperature test is designed based on product characteristics. In this study, gels were prepared and stored at a temperature of 4℃ and 40ºC, and the temperature was changed every 24 hours for seven days, simulating extreme storage conditions.[32] The organoleptic result is presented in Table 15, and changes in the pH, viscosity, spreadability, and Stickiness tests are presented in Table 16. The organoleptic test showed no changes in colour, odour, or texture. However, there were changes in air bubbles that increased as storage was carried out at two different temperatures and as a result of moving the container when testing was carried out. Meanwhile, there was a shift in pH, viscosity, spreadability, and stickiness. During storage at 4ºC and 40ºC for six cycles (12 days), there was a decrease in pH and spreadability, but the viscosity and Stickiness increased (Table 16).

Table 16: pH, viscosity, spreadability, and adhesion tests of the optimum formula for six cycles

Cycle	pH	Viscosity (cP)	Spreadability (cm)	Stickiness (seconds)
	Mean ± SD			
0	6.31± 0.01	14506.67 ± 272.27	5.40 ± 0.32	4.59 ± 0.32
1	6.28 ± 0.01	14813.33 ± 526.24	5.39 ± 0.09	4.72 ± 0.18
2	6.25 ± 0.02	15100 ± 105.83	5.27 ± 0.12	6.56 ± 2.97
3	6.21 ± 0.04	15353.33 ± 378.59	5.25 ± 0.18	6.85 ± 2.45
4	6.20 ± 0.005	15453.33 ± 456.22	5.24 ± 0.16	7.13 ± 2.86
5	6.17 ± 0.02	15720 ± 52.91	5.17 ± 0.10	7.69 ± 3.80
6	6.15 ± 0.01	15933.33 ± 64.29	5.10 ± 0.09	9.52 ± 2.31



(a) (b) (c)

Figure 3: Model graph of component factor relationship with response (a) pH, (b) viscosity, (c) spreadability

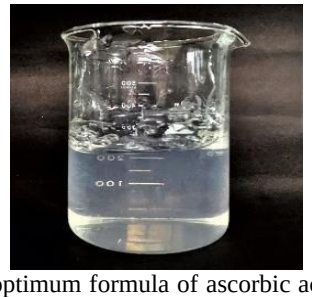


Figure 4: The optimum formula of ascorbic acid transfersome asam ascorbate

Changes in pH values can be influenced by opening the container during testing, where carbon dioxide (CO2) is trapped in the gel preparation. pH can be closely related to dissolved carbon dioxide (CO2), which participates in equilibrium reactions (acidification process). Carbon dioxide (CO2) in the air enters and mixes into the gel preparation and reacts with water (H2O), the solvent that has the most significant content in the gel, and then forms carbonic acid (H2CO3). Carbonic acid (H2CO3) in water will release H+ ions and form bicarbonate (HCO3-), and bicarbonate ions (HCO3-) will release H+ ions and form carbonate (CO32-) so that the released H+ ions cause the pH of the gel preparation to decrease.[37] In addition, the decrease in pH can also be caused by preparations that experience cation hydrolysis from TEA, which is a weak base, by producing H+ ions so that the pH of the gel preparation becomes more acidic or decreases with storage.[42] Although there was a decrease in the pH of the gel preparation during storage, the decrease is relatively stable and still within the skin pH range, which is 4.5-6.5.[23] The increase in viscosity during storage is partly due to water loss that evaporates from the gel preparation due to treatment at two different temperatures. When the gel is heated, there will be a random arrangement of polymers in the preparation, while when cooling, double helical chains will form continuous cross-links from a matrix. The more the number of bonding zones, the more the release of water increases because the formation of helical bonds and aggregates will narrow the voids between the bonds so that free water is released from the gel, increasing the viscosity of the preparation during storage. [43] A slight change in viscosity value is considered relatively stable because of the wide range of requirements of viscosity values. Viscosity values range of 2000–50000 cPs is considered good.[25]

Conclusion

The formulated ascorbic acid transferosomes showed good characteristic results from the particle size requirements, polydispersity index value, and zeta potential values obtained in this study. These parameters were in the acceptable range. The optimum formula of ascorbic acid transferosome gel produced using the D-Optimal Design method has a carbopol 940 concentration of 0.8% and propylene glycol of 3.7% with a desirability value of 1. The optimum formula of ascorbic acid transferosomes gel also exhibited good stability using the cycling test method for six cycles. The formulated gel holds the potential of a good cosmeceutical.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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