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Optimization and Characterization of a Salicylic Acid-Aloe Vera Transethosome Dispersion for Topical Anti-Acne Application

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Abstract

Transethosomes are new nanovesicular carriers made of phospholipids, ethanol, surfactants, and co-surfactants that help drugs penetrate through the stratum corneum. Salicylic acid and Aloe vera are well-known anti-acne agents. Combining both in a transethosomal system is expected to improve their effectiveness against acne vulgaris. This study aimed to develop and optimize a salicylic acid and Aloe vera transethosome dispersion as a nanocarrier system for topical anti-acne use, utilizing a Simplex Lattice Design (SLD) approach. Aloe vera gel concentrate (KGLB) was created from fresh gel through freeze-drying and was characterized by phytochemical screening. We evaluated its antibacterial activity against Propionibacterium acnes (PA) and Staphylococcus epidermidis (SE) using the agar well diffusion method. Optimization involved changing the concentrations of lecithin, ethanol (96%), Tween 80, and propylene glycol, while keeping salicylic acid (0.5% w/v) and KGLB (12.5% w/v) at fixed levels throughout the formulation process. Particle size, polydispersity index (PDI), and zeta potential were used as response variables. The SLD optimization found the best transethosomal composition to be 1.5% lecithin, 40% ethanol, 4% Tween 80, and 2% propylene glycol, with a desirability value of 0.868. This formulation had a mean particle size of 48.96 ± 6.77 nm, a PDI of 0.23 ± 0.02, and a zeta potential of -31.6 ± 6.85 mV. Transmission Electron Microscopy (TEM) analysis confirmed the presence of spherical nanovesicles, and FTIR spectra showed hydrogen-bond interactions without chemical incompatibility. Phytochemical screening identified saponins, flavonoids, glycosides, tannins, and steroids in KGLB. The combination of salicylic acid and KGLB demonstrated strong antibacterial activity against both PA and SE, with salicylic acid being the main contributor. Stability assessments over 12 weeks at 4 °C and 28 °C revealed acceptable physicochemical characteristics; however, higher temperatures (40 °C) caused the particle size to increase, indicating temperature sensitivity. In conclusion, the salicylic acid and Aloe vera transethosome dispersion was successfully optimized using SLD, showing strong antibacterial activity, suitable nanophysical properties, and good stability. These results support its potential use in a topical formulation for acne treatment.

Keywords: Acne vulgaris, Aloe vera, Nanovesicle, Salicylic acid, Simplex lattice design, Transethosome

Introduction

Acne vulgaris is a chronic inflammatory skin disorder that affects both adolescents and adults. It is characterized by comedones, papules, pustules, nodules, and cysts, primarily on the face, back, and chest. While not life-threatening, acne can substantially reduce self-confidence and frequently results in permanent scarring(1). Globally, acne vulgaris is among the most prevalent dermatological conditions, affecting approximately 9-10% of the global population and ranking within the ten most common diseases worldwide(2). In Indonesia, the prevalence of acne is 80-85% among adolescents, 12% in women over 25 years, and about 3% in individuals aged 35–44 years(3). The pathogenesis is primarily linked to *Propionibacterium acnes* (now classified as Cutibacterium acnes), which induces inflammation in pilosebaceous follicles, and Staphylococcus epidermidis, which can cause abscesses in cases of secondary infection(4).

Topical agents are among the most effective ways to manage acne. However, their limited ability to penetrate the skin often lowers their effectiveness. To address this issue, drug delivery systems based on nanotechnology, like ethosomes

and transethosomes, have been created. Transethosomes, a modified version of ethosomes, consist of phospholipids, ethanol, and an extra surfactant. This combination improves the delivery of active compounds into deeper layers of the skin[5]. Compared to traditional liposomes, ethosomes offer better transdermal delivery with less toxicity[6]. To optimize transethosomal formulations, researchers can use statistical methods like the Simplex Lattice Design. This method helps identify the most effective composition ratios[7]. Previous research has shown that the concentrations of ethanol, phospholipid, and surfactant are key in creating stable nanovesicles that are small enough for effective intradermal penetration[8].

Salicylic acid and Aloe vera are just two of the possible anti-acne ingredients that would be an interesting combination to be used in a transethosomal vehicle. SA has combined keratolytic and antibacterial effects on both *P. acnes* and *S. epidermidis*, especially when dissolved in nanogel systems[9]. Moreover, Aloe vera gel is rich in over 75 bioactive ingredients such as saponins, anthraquinones and acemannan which have anti-inflammatory, antibacterial, and wound-healing properties[10,11]. Despite the fact that both agents are identified as good anti-acne medications, no much information is available about their joint application into a transethosomal carrier. According to the promoted penetration efficiency of transethosomes, it is anticipated that salicylic acid in combination with Aloe vera extract would give synergistic antibacterial and antiinflammatory effects. Accordingly, the purpose of the present work was to prepare, evaluate and optimize transethosomal formulation for salicylic acid and Aloe vera which could be taken as more efficient topical treatment against acne vulgaris.

Materials and Methods

Study Design

The research followed an experimental study design in which effects of predetermined treatments could be tested under the manipulation manageable conditions (things that researchers can control), where the observations could be measured quantitatively and statistically analysed[12]. The research included four major phases: (a) antibacterial activity estimation, (b) transethosomal formulation optimization, (c) physical characterization of the drug-loaded nanovesicles, and (d) in vivo evaluation of the optimized transethosome formulation.

Materials

The applied active ingredients were: salicylic acid (Sigma Aldrich, USA) and gel concentrate of Aloe vera (KGLB), obtained from the fresh aloe vera leaves. Other excipients included soy lecithin (Novio, Indonesia), Tween 80 (Merck, Germany), ethanol (96%), propylene glycol, and distilled water. Bacterial strains Propionibacterium acnes and Staphylococcus epidermidis were obtained from the Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. Nutrient agar, nutrient broth, and Mueller Hinton Agar were used for bacterial culture. All other reagents (analytical grade) were procured from recognized suppliers.

Instruments

The instruments used in this study included standard laboratory glassware (Iwaki Pyrex®, Japan), a magnetic stirrer and hot plate (Velp Scientific®, Italy), micropipettes (Eppendorf®, Germany), refrigerator, freeze dryer (BK-FD10P, Biobase®, China), pH meter (Universal Instrument®, USA), analytical balance (Kern®, Germany), UV–Vis spectrophotometer (Shimadzu®, Japan), centrifuge (EBA 21, Hettich®, Germany), particle size analyzer (Litesizer™ 500, Anton Paar®, Austria), laminar air flow cabinet (HL 36Ae®, Indonesia), oven (Memmert®, Germany), autoclave (HL 36Ae®, Indonesia), FTIR spectrophotometer (Thermo Scientific®, USA), vortex mixer, ultrasonic sonicator (Branson®, USA), ultra-homogenizer (IKA®, Germany), incubator (Memmert®, Germany), and Transmission Electron Microscope (TEM, JEOL®, Japan).

Plant Material and Preparation of Aloe Vera Gel Concentrate

Fresh aloe vera leaves were collected from Tanjung Morawa, North Sumatra, Indonesia. The plant was authenticated at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. The leaves were washed, peeled, and the inner gel was scraped, blended, and filtered using a 60-mesh sieve. The filtrate was then subjected to freeze-drying for 36 h to obtain the Aloe vera gel concentrate (KGLB) in the form of a dry powder. For subsequent experiments, the freeze-dried KGLB powder was reconstituted in distilled water prior to use. All KGLB concentrations reported (12.5% w/w, for example) are w.w.)[13,14].

Phytochemical Screening

Preliminary phytochemical screening of KGLB for the presence of secondary metabolites including alkaloids, flavonoids, saponins, tannins steroids and glycosides was carried out using standard qualitative methods.

Antibacterial activity

Antibacterial activity assessment the test against bacterial strains was assessed by the well diffusion method. Bacterial suspensions of *P. acnes* and *S. epidermidis* were suspended in 0.9% NaCl solution to a 0.5 McFarland (≈1.5 × 108

CFU/mL). Mueller Hinton Agar plates were inoculated with bacterial suspensions, and wells were filled with test samples including: (a) salicylic acid solutions at concentrations of 0.125-1% (w/v); (b) Aloe vera gel concentrate (KGLB) solutions at concentrations of 6.25-50% (w/v); (c) a salicylic acid-KGLB mixture containing 0.5% (w/v) salicylic acid and 12.5% (w/v) KGLB (weight ratio 1:25); (d) clindamycin 0.1% (w/v) as a positive control; and (e) 10% (v/v) DMSO as a negative control. All samples were prepared in distilled water unless otherwise stated. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured using a digital caliper. All antibacterial assays were performed in triplicate (n = 3) to allow statistical analysis.

Optimization of Transethosome Formulation

Optimization was carried out using Simplex Lattice Design (Design Expert®) with salicylic acid and KGLB as active agents. The independent variables were lecithin (0.5-1.5%), Tween 80 (4-5%), ethanol (39-40%), and propylene glycol (2%). The concentration of ethanol was chosen relatively low as around 40%, which is believed to increase the vesicle flexibility and ability of drug permeation without affecting transethosome stability, higher concentrations showed that it could disrupt the bilayers of phospholipids[15]. The dependent responses were particle size, polydispersity index (PDI), and zeta potential. The optimum formulation was selected based on the desirability function.

Preparation of Transethosomes

The cold method for preparing the optimized formulation was used as described by the references [16,17]. Holtz method was described as follows: Lecithin was dispersed in Tween 80 using a magnetic stirrer at 30 °C for 10 min at 750 RPM. Then, propylene glycol was added gradually, and the system was stirred for another 10 min to achieve a homogeneous mass (Mass 1). In a separate mass, salicylic acid was dissolved in 96% ethanol, and KGLB was added to otain a clear solution (Mass 2). Mass I was added all at once to Mass II and stirred for another 10 min at 30 °C and 750 RPM. After the 10 min, distilled was added, and the dispersion was stirred for an additional 15 min to produce the transethosomal vesicular system. The vesicle size was optimized by high-speed homogenization in an ultra-homogenizer, followed by a 15 min sonication. The formulation was then refrigerated until characterization was performed [17].

Characterization of Transethosomes

The optimized salicylic acid–Aloe vera transethosome formulation was subjected to in-vitro anti-acne activity evaluation using an antibacterial assay against Cutibacterium (Propionibacterium) acnes and Staphylococcus epidermidis. The vesicle shape was analyzed through Transmission Electron Microscopy (TEM), and the potential chemical interactions between the components of the formulation were analyzed using Fourier Transform Infrared (FTIR) spectroscopy[18]. For the preparatory topical formulation, the required organoleptic, physicochemical, and rheological characterization included organoleptic evaluation, assessment of homogeneity, determination of pH, evaluation of viscosity, and assessment of the physical consistency and ease of handling of the transethosomal dispersion [19]. All characterization experiments were performed in triplicate (n = 3) and the results were subjected to statistical analysis.

Results and Discussion

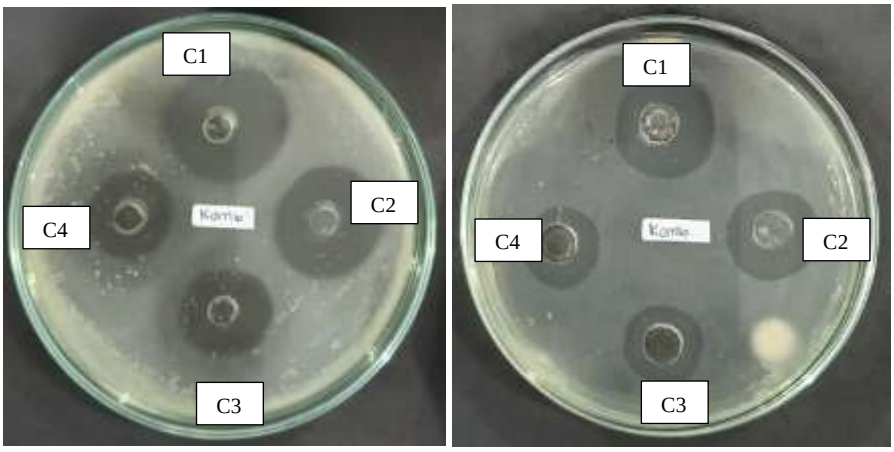
The first step of the yield analysis was the processing of the fresh Aloe vera gel into Aloe vera gel concentrate (KGLB) through the method of freeze drying. The chosen method was freeze drying as it reduces the water content, while the aroma, color, and other organoleptic properties are retained [20]. The yield was calculated through the mass after treatment (g) versus the mass prior to treatment (g). The pre-processing mass was aloe vera leaves that had been blended while the post-processing mass was the aloe vera jelly that had been freeze-dried. The yield was calculated to assess the efficiency of the processing method and to quantify the amount of material retained post-processing, expressed as a percentage (%). The extraction yield was 62.66%. The higher the yield value, the more natural constituents have been extracted, indicating more efficient treatment and higher bioactive component content. Freeze-drying is a method of non-thermal drying where water is removed at low temperatures, and it has several advantages; it reduces shrinkage and other structural changes, accelerates the water removal process, and is able to preserve nutrients while causing little change to the aroma, taste, and color[21].

Phytochemical investigation showed that the aloe vera jelly concentrate (KGLB) consists of saponins, flavonoids, glycosides, tannins, and steroids. The solubility of various phytochemicals is determined by the polarity of the solvents. Polar phytochemicals dissolve in polar solvents while non-polar phytochemicals dissolve in non-polar solvents. Saponins, which are glycosidic in nature are amphiphilic and have the capability of forming micelles and generating foams in aqueous solutions[22,23].The presence of flavonoids was tested by the Mg–HCl test, which is known for hydrolyzing O-glycosides to aglycones and producing a red or orange color upon reduction[24]. Glycosides are sugar and nonsugar compound

consisting of a sugar moiety and a non-sugar moiety, which reacts with α – naphthol and concentrated sulfuric acid to form a purple ring due to the condensation of f urfural derivatives[25]. The presence of tannins was ascertained by using FeCl3, which gave a greenish-black complex, while steril hated phytochemicals are classified as polyhydroxy phenols that are able to precipitate proteins. The presence of steroids was determined by the Liebermann–Burchard test, in which oxidation gave a blue and green color due to the formation of conjugated double bonds. Steroids are among the major secondary metabolites along with phenolics, terpenoids, essential oils, alkaloids, and polypeptides [26].

Antibacterial activity against P. acnes and S. epidermidis was evaluated based on inhibition zone assays. Clear inhibition zones were produced by both salicylic acid and KGLB extracts. Higher concentrations of KGLB and salicylic acid produced larger zones of inhibition. Salicylic acid at 0.5% (w/v) produced the highest zone of inhibition of 25.83 ± 2.89 mm against P. acnes and 20.33 ± 0.21 mm against S. epidermidis. KGLB also produced strong inhibition at 12.5% (w/v) with corresponding zones of 17.87 ± 0.90 mm and 16.33 ± 0.38 mm, respectively. The most enhanced antibacterial effect was produced by the combination of salicylic acid and KGLB. The best combination was 0.5% salicylic acid with 12.5% KGLB with zones of 25.37 ± 0.72 mm against P. acnes and 20.83 ± 0.50 mm against S. epidermidis.

A one-way ANOVA with post hoc testing showed that there was no difference ($p > 0.05$) between the antibacterial activity of salicylic acid alone and the combination formulation, though both were significantly more effective than KGLB alone ($p < 0.05$). These observations suggested that salicylic acid may exert the predominant antibacterial effects and KGLB could be acted as secondary compound in the mixture. Antibacterial activity of the oil against P. acnes was highly significant. The antibacterial effects observed might be due to additive properties of salicylic acid as a bacteriostatic agent[27] in combination with aloe-derived chemical compounds like the anthraquinones[28]. DMSO, a negative control did not exhibit antibacterial activity indicating that the inhibition was exerted by the test agents but not from the solvent[29]. Clindamycin 0.1% as a positive control showed the greatest activity with zone of inhibition of 48.5 ± 1.11 mm to P. acnes and 47.9 ± 0.30 mm against S. epidermidis (results are expected due to its established effect on Gram-positive organisms)[30]. The antibacterial activity data are also shown in Figure 1.



(A) P. acnes **(B) S. epidermidis**

Figure 1. Inhibition zone diameters of combination formulations salicylic acid and KGLB against (A) *Propionibacterium acnes* and (B) *Staphylococcus epidermidis*. Combination formulations consisted of salicylic acid with 12.5% KGLB: C1 (1%), C2 (0.5%), C3 (0.25%), and C4 (0.125%)

Optimization of Salicylic Acid-Aloe Vera Transethosome Formulation

The best formulation was obtained by the Design Expert® software employing Simplex Lattice Design. Such a design would be more appropriate for mixture formulation systems as it studies the effect of component proportions rather than absolute concentrations which is not possible to optimise via RSDs like BBD or CCD [31]. Furthermore, SLD offer efficient formulation space exploration based on few experimental runs and preserves good prediction accuracy for mixture optimization[32]. Twelve different compositions (runs) were obtained for various proportions of three composition factors, lecithin, ethanol (96%), and Tween 80; the proportion of propylene glycol was maintained at 2% constituting the starting composition unchanged in all formulations. The compositions of the formulations were detailed as shown in Table 1. Optimization results including particle size, polydispersity index (PDI), and zeta

potential are presented and discussed separately in the following section.

Table 1. Composition of Transethosome Formulation Using Simplex Lattice Design

Run	Lecithin (%)	Ethanol 96% (%)	Tween 80
1	1.5	40.0	4.0
2	1.2	39.7	4.7
3	0.5	40.0	5.0
4	1.5	39.5	4.5
5	1.5	39.5	4.5
6	1.0	40.0	4.5
7	1.0	39.5	5.0
8	1.0	39.5	5.0
9	1.0	40.0	4.5
10	1.5	39.5	4.5
11	1.0	40.0	4.5
12	1.5	39.0	5.0

Lecithin, the main structural component, functions as a vesicle-forming agent. Ethanol enhances skin penetration by fluidizing the lipid bilayer. Tween 80, a non-ionic surfactant, was incorporated as an edge activator to improve vesicle deformability and flexibility, which consequently influences drug permeability [33]. However, the use of a single surfactant is often insufficient to reduce interfacial tension; therefore, a co-surfactant was included to further improve flexibility and reduce the energy required for globule disruption, leading to smaller particle size [34,35]. Propylene glycol was added as a co-solvent due to its low toxicity and its ability to enhance the solubilization of hydrophilic surfactants within the lipid base, thereby improving formulation stability.

Characterization of Optimization of Salicylic Acid-Aloe Vera Transethosome Formulation

Optimization of the salicylic acid-Aloe vera transethosome (KGLB) formulation was performed using Design Expert® software (version 13) employing a Simplex Lattice Design (SLD). This design is particularly appropriate for mixture-based formulations, in which the proportions of formulation components are constrained to a fixed total and the responses depend on the relative ratios rather than absolute quantities. The effects of lecithin (A), ethanol 96% (B), Tween 80 (C), and propylene glycol (D) on key physicochemical responses: particle size, polydispersity index (PDI), and zeta potential were evaluated. Model adequacy was assessed using analysis of variance (ANOVA), determination coefficients (R^2, adjusted R^2, and predicted R^2), and lack-of-fit tests. All selected models were statistically significant ($p < 0.05$) with non-significant lack of fit ($p > 0.05$), indicating good agreement between predicted and experimental data. Based on the desired attributes of a stable nanovesicular system for topical delivery, optimization criteria were established as shown in Table 2.

Table 2. Optimization Criteria for Salicylic Acid-Aloe Vera Transethosome

Response Parameter	Lower Limit	Upper Limit	Optimization Goal
Particle size (nm)	100	500	Minimize
Polydispersity index	0.00	0.50	Minimize
Zeta potential (mV)	-60	+60	Maximize

Based on the desirability function approach, the optimum transethosome formulation consisted of 1.5% lecithin, 40% ethanol (96%), 4% Tween 80, and 2% propylene glycol, yielding an overall desirability value of 0.868, indicating that the optimization criteria were satisfactorily achieved. The predicted responses for the optimized formulation were: Particle size: 262.9 nm; Polydispersity index: 0.265; and Zeta potential: –30.5 mV. The desirability profile and superimposed contour plots are illustrated in Figures 2 and 3, respectively.

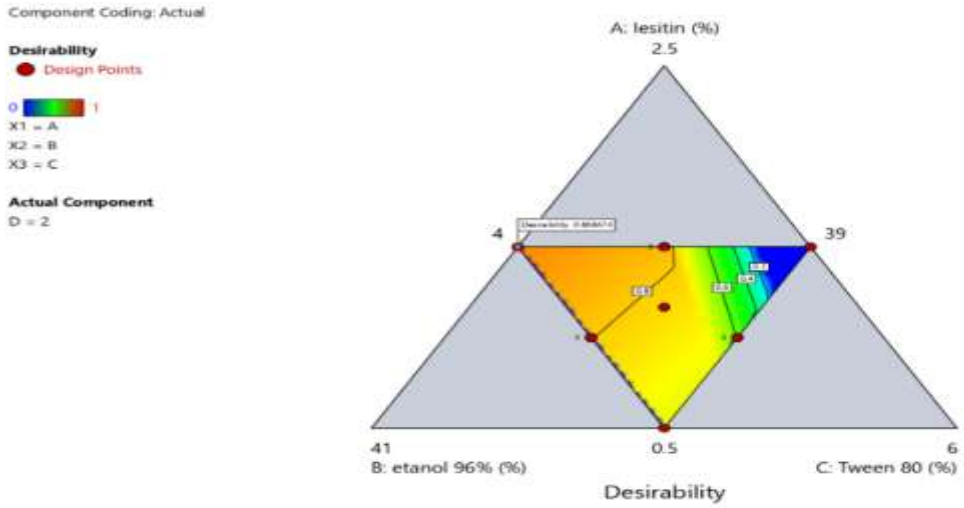


Figure 2. Desirability graph of optimum formula of transethosome salicylic acid-KLJB

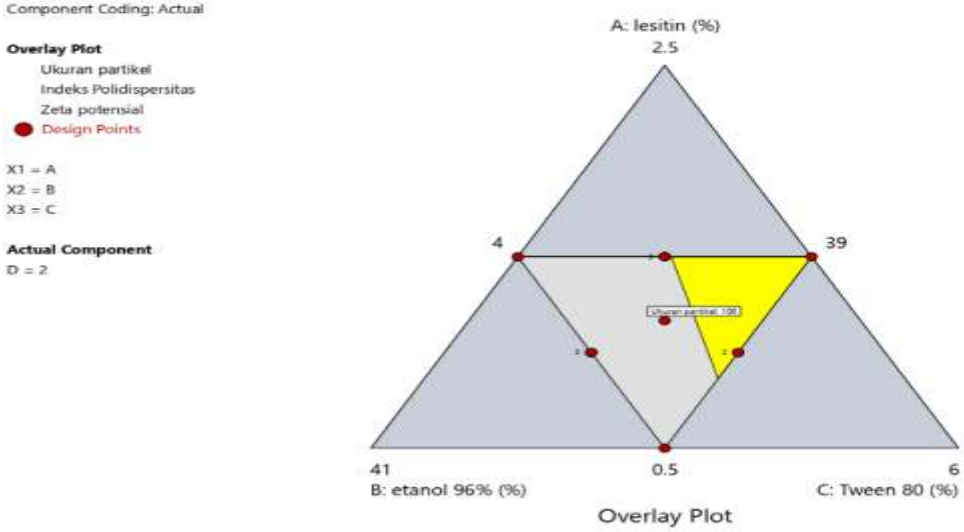


Figure 3. Superimposed contour plot of optimum formula of transethosome salicylic acid-KLJB

The solution and prediction of the optimum formula of salicylic acid transethosome with KGLB obtained from Simplex Lattice Design are presented in Table 3.

Table 3. Predicted Optimum Transethosome Formula

Lecithin (%)	Ethanol 96%	Tween 80 (%)	Propylene glycol (%)	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Desirability
1.5	40	4	2	262.9	0.265	-30.5	0.868

To validate the predictive capability of the model, the predicted values were compared with experimental results using a one-sample t-test in SPSS Statistics®, as shown in Table 4.

Table 4. Verification of Optimum Formula

Response	Predicted	Actual	*p Value*	Remark
Particle size (nm)	262.9	48.96 ± 6.77	0.000	Significant difference
Polydispersity index	0.265	0.23 ± 0.02	0.740	Not significant
Zeta potential (mV)	-30.5	–31.6 ± 6.85	0.740	Not significant

The particle size of the optimal transethosome was significantly different in predicted (262.9 nm) and measured (48.96 ± 6.77 nm) values ($p < 0.05$). The actual particle size was much smaller than expected, which is beneficial for the purpose of dermal delivery since it falls well within the nanovesicle range (<100 nm), allowing better penetration into skin and less discrepancy with the targeted dimension. However, there were no significant differences between predicted and experimental values for polydispersity index and zeta potential and experimental values ($p > 0.05$), validating the robustness of the model. The results indicate that SLD is a reliable approach in optimization and prediction of physical properties of the formulation. The optimized transethosome with desirability value of 0.868 showed good physical properties as liquid dispersion. In general, the optimization procedure was fruitful and gave results consistent with that of other work which suggested Simplex Lattice Design to be potent and reproducible[32].

Morphological Results of Salicylic Acid Transethosomes with Optimum KGLB

The morphology of Salicylic acid–KGLB transethosomes was viewed under Transmission Electron Microscopy (TEM). TEM is mainly used for the information about vesicle shape and surface appearance[36]. The study sample (yellow dispersion) was analyzed by TEM for morphology. The micrographs of TEM are shown in Figure 4.

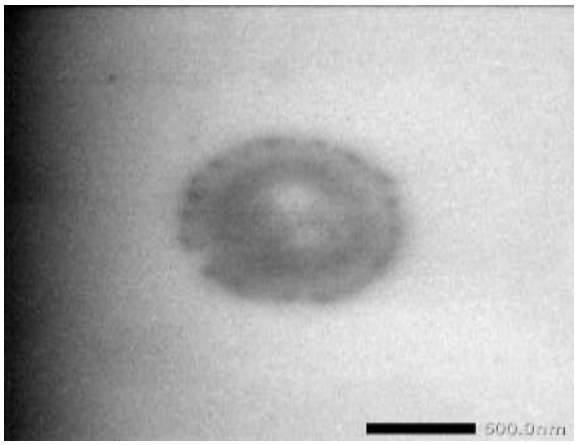


Figure 4. TEM images of salicylic acid–KGLB transethosomes. The TEM results revealed that the salicylic acid–KGLB transethosomal vesicles were spherical. Spherical vesicular form is an ideal one which helps for the ease of penetration through stratumcorneum[37]. Variations in size measured using TEM and DLS are frequently reported for vesicular nanocarriers. DLS measures the hydrodynamic diameter of vesicles in a hydrated and scattered state, consisting of the vesicle core, lipid bilayer and surrounding layer of hydration, thereby contributing to larger recorded measurements [40]. TEM, on the other hand, is performed under vacuum and with dried samples lacking in hydration layer leading to vesicle shrinkage in the process of preparing sample. Therefore, TEM only reflects the real physical structure and morphologies of vesicles, while DLS is a better estimate of vesicle size distribution in sol system.

FT-IR Analysis of Optimized Salicylic Acid–Aloe Vera Transethosomes

The FT-IR spectra showed characteristic absorption bands attributed to functional groups, peak intensity and wavenumber assigned in %T. These peaks are due to two factors, the molecular vibration relates to energy interactions and various functional groups have different bonds and Table 1 IR of its absorption [38]. To study any chemical interaction between salicylic acid, Aloe vera (KGLB) and excipients in the optimized transethosome formulation, FT-IR analysis was carried out. The salicylic acid, KGLB and optimal trnasethosome formulation are shown in Figure 5 and Table 5.

Table 5. FT-IR Spectral Analysis of Salicylic Acid, Aloe vera (KGLB), and Optimized Transethosomes

Wavenumber (cm^{-1})				Functional Group	Interpretation
Salicylic Acid	KGLB	Salicylic Acid Transethosome with Aloe Vera Gel	Reference Range (cm^{-1})		
3324.74	3324.74	3341.97	3550–3200	O–H stretching	The blue shift of the O–H stretching band from 3324 to 3341 cm^{-1} suggests altered

Continued table 5.

					hydrogen-bonding interactions associated with encapsulation in the transethosomal matrix
2945.75–2830.91	–	2951.50–2848.14	2960–2850	C–H stretching	The observed shift indicates the stability of the C–H bond within the system.
2360.05–2337.08	2360.05	2360.05–2337.08	2400–2300	CO_2	Possible residue of solvent or atmospheric CO_2.
1665.76–1510.20	1636.53–1544.65	1648.01–1510.20	1700–1500	C=O (COO^-) asymmetric and symmetric stretching; aromatic C=C	Shifts indicate interactions between carboxylate groups and compounds present in Aloe Vera Gel.
1452.78–1303.48	1475.75–1314.97	1458.52–1360.90	1500–1300	C–O (phenolic), O–H bending	Shifts suggest interactions involving OH groups.
1240.32–1022.11	1286.25–1148.44	1297.74–1016.37	1300–1000	C–O–C stretching	Shifts indicate interactions of ether/ester groups.

In general, FT-IR spectra verified that hydrogen bonding was formed between salicylic acid and KGLB components in the optimal transethosome. This interaction does not evidence new chemical bond development but improves the stability of the formulation, suggesting an excellent salicylic acid–carrier matrix physical compatibility.

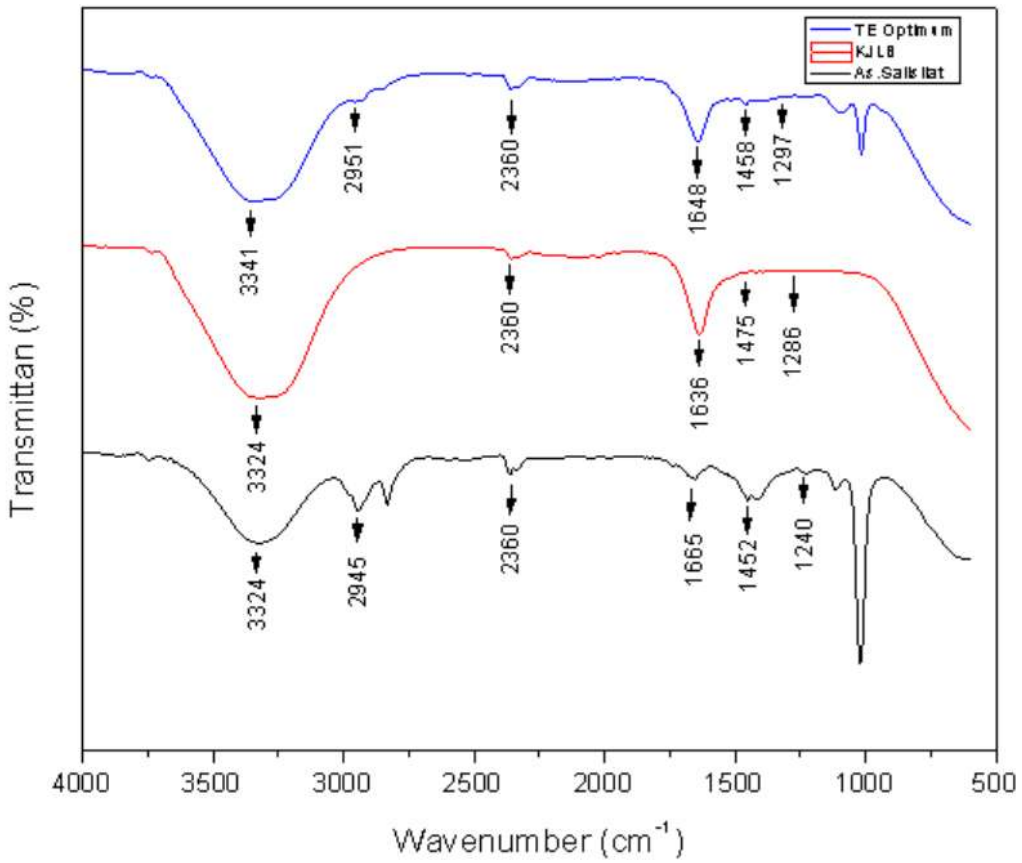


Figure 5. FT-IR spectra of salicylic acid, Aloe vera (KGLB), and optimized transethosomes

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Characterization of optimized salicylic acid–aloe vera transethosomes

The prepared optimum salicylic acid–Aloe vera transethosome formulation was subjected to organoleptic characters evaluation, homogeneity, pH value, viscosity, particle size analysis, PDI and Zeta potential. The summary of the results is presented in Table 6

Table 6. Physical characteristics of optimized salicylic acid–Aloe vera transethosomes

No.	Parameter	Observation	Mean ± SD
1	Organoleptic	Yellow color, clear solution, and odourless	–
2	Homogeneity	Homogeneous	–
3	pH	–	4.97 ± 0.01
4	Viscosity	–	6152 ± 942.5 cPs
5	Particle size	–	48.96 ± 6.77 nm
6	PDI	–	0.23 ± 0.02
7	Zeta potential	–	-31.6 ± 6.85 mV

The transethosome formulation was found to be a clear, yellow and odorless solution as judged by visual examination. Yellow coloration was due to the dispersion of lecithin and Tween 80. The formulation was found to be homogenous in appearance with no observable large particles, with color being evenly distributed on visual inspection which suggested good distribution of the active within the MVS. The solution's pH value (4.97 ± 0.01) fell between the normal physiological skin range of 4.5–6.5, indicating that it was safe for topical use. (h) pH stability within this range ensures that the preparation is not so acidic or alkali as to cause irritation[39]. The viscosity (measured using a Lamy Rheology viscometer) was 6152 ± 942.5 cPs (within accepted range to SNI 16-4399-1996 Indonesian National Standard for topical preparations) making it suitable for dermal application. The size of the nanovesicles was also demonstrated by DLS analysis, which showed an average particle size of 48.96 ± 6.77 nm, confirming that the particles form a vesicle and belong to the nanovesicular size (<1000 nm). Sub-600 nm nanovesicles are said to be capable of better skin penetration. The PDI was found to be 0.23 ± 0.02, which implies a polydisperse system. This value indicates heterogeneity in particle size distribution, according to the literature, PDI <0.3 are typically acceptable for nanocarrier formulations (indicating good uniformity in the particle size distribution)[40]. The average zeta potential of the transethosomal preparation was –31.6 ± 6.85 mV, which means good colloidal stability of the system. For colloids, zeta potential above +30 mV or below -30 mV, high stability are observed as a result of enough electrostatic repulsion[41]. In conclusion, the optimized salicylic acid, Aloe vera transethosome fulfilled the general criteria of nanovesicle systems in particle size, viscosity, pH and homogeneity and colloidal stability. In order to enhance stability and application comfort, additional formulation into a gel base is preferred.

Stability evaluation of optimized Salicylic acid–Aloe vera transethosomes

The stability profile of the optimized salicylic acid Aloe vera transethosome formulation over a 12-week period under different storage temperature conditions is shown in Table 7.

Table 7. Stability profile of optimized salicylic acid–Aloe vera transethosomes under different storage conditions

Parameter	Week	28 ± 2 °C	4 ± 2 °C	40 ± 2 °C
Organoleptic	0–12	Yellow; Clear; Odorless	Yellow; Clear; Odorless	Yellow; Clear; Odorless
Homogeneity	0–12	Homogeneous (+)	Homogeneous (+)	Homogeneous (+)
pH	0	4.97 ± 0.01	4.97 ± 0.01	4.97 ± 0.01
	12	5.61 ± 0.08	5.46 ± 0.10	6.02 ± 0.02
Viscosity (cPs)	0	6152 ± 942.5	6152 ± 942.5	6152 ± 942.5
	12	7852 ± 407.9	8874 ± 340.4	7509 ± 50.9
Particle size (nm)	0	48.96 ± 6.77	48.96 ± 6.77	48.96 ± 6.77
	12	21.67 ± 0.74	67.32 ± 1.35	227.41 ± 164.96
Polydispersity Index (%)	0	0.23 ± 0.02	0.23 ± 0.02	0.23 ± 0.02
	12	0.25 ± 0.01	0.25 ± 0.03	0.25 ± 0.02
Zeta potential (mV)	0	-31.6 ± 6.85	-31.6 ± 6.85	-31.6 ± 6.85
	12	-18.77 ± 9.00	-14.53 ± 2.63	-21.70 ± 5.59

The optimized formulation of salicylic acid–aloe vera transethosome possessed uniform and stable organoleptic characters as well as homogeneous content during the 12 weeks study period, irrespective of storage method applied which confirms its physical integrity. The pH oscillated slightly, but within the acceptable range for skin (4.5–6.5), in agreement with making their use safe for topical application, although there was a steady increase to 6.02 at 40 °C pointing out to a temperature induced drift. Viscosity values were higher in all formulations, being significantly more stable at 4 °C; however, they remained within the desirable limits for pharmaceutical topical preparations. Size changes of particles were temperature dependent, with a decrease at 28 °C (21.67 nm), a moderate increase at 4 °C (67.32 nm) and an appreciable enlargement at 40 °C (227.41 nm) suggesting possible aggregation at higher temperature. The PDI has also increased only slightly under all the storage conditions, demonstrating a modest polydispersity of particle size. At Week 0, zeta potential (−31.6 mV) illustrated an initial colloidal stability of the transethosomal system. However, with storage there was a slow increase in the zeta potential towards less negative (about −18 to −21 mV) reflecting lower electrostatic repulsion and poor colloidal stability of aged samples. The stability results were descriptively analyzed as the mean ± S.D in order to check for trends on physico-chemical changes during storage. The primary focus of the stability study was not to perform inferential statistical tests between storage groups; but rather, to provide information concerning the physical integrity of the product over various temperature conditions. In general, most physicochemical properties were within acceptable limits with respect to topical vesicle systems, confirming that the formulation structure was maintained; however high temperature (40 °C) promoted an increase in vesicle size and the instability of colloidal dispersion.

There are, however, some limitations in the current study: one of them is not having a release study "in vitro" to learn about the release profile of salicylic acid from transethosome system. An in vitro release study is important to reveal the drug-release mode of vesicular carriers as well as its link with skin penetration profile. Hence, in future the in vitro release profile will be investigated by using diffusion cell method to have broader perspective toward delivery performance of this system. Rain tapped softly on the window, keeping time with the hush inside. Everything outside faded into smudgy gray, but the fireplace glowed and wrapped the room in warmth. I sank into my favorite chair, book in hand, flipping pages one by one and letting each sentence draw me in. Time seemed to pause like the rain built a little world just for me. Right then, all that really mattered was the story in my hands and that calm, quiet feeling drifting through the house.

Conclusion

This study found that salicylic acid and Aloe vera Gel Concentrate (KGLB) both stop the growth of Propionibacterium acnes and Staphylococcus epidermidis. When mixed into the transethosome formula, neither ingredient reacted with the other components, so the formula stayed chemically stable. The optimized transethosome held up well at both fridge temperature (4 °C) and room temperature (28 °C). But things changed at higher heat at 40 °C, the particles got bigger, which means the colloidal stability dropped and the vesicles started clumping together. Clearly, this formula doesn't like heat and needs to be kept cool. Also, everything here is based on lab tests the work focused on antibacterial activity and stability, nothing more. The formulation was not evaluated in the form of a gel, nor were in vivo efficacy and long-term stability tests conducted. Therefore, the findings should be interpreted with caution, and further studies are required to confirm clinical relevance. Future studies are recommended to formulate salicylic acid and Aloe vera Gel Concentrate into a transethosomal gel preparation. Subsequent research should also evaluate the antibacterial efficacy of the gel formulation through in vivo or clinical studies and conduct comprehensive stability testing under various storage conditions to ensure product safety and effectiveness.

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Conflicts of Interest

There is no conflict of interest regarding the publication of this work.

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Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Minda Sari Lubis, Julia Reveny, Poppy Anjelisa Zaitun Hasibuan, Anayanti Arianto; data collection: Minda Sari Lubis; analysis and interpretation of results: Julia Reveny, Poppy Anjelisa Zaitun Hasibuan, Anayanti Arianto; draft manuscript preparation: Minda Sari Lubis, Julia Reveny. All authors reviewed the results and approved the final version of the manuscript.

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