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Optimization of Phosphatidylcholine and Tween 80 Composition in the Formulation of Icariin Transfersome as a Transdermal Delivery System using Design-Expert

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ABSTRACT

Icariin is the main flavonoid of Epimedium sp. and has phosphodiesterase type-5 inhibitor (PDE5I) activity; however, its low water solubility and membrane permeability limit its oral bioavailability. The transferosome-based transdermal drug delivery system approach is considered promising because it can increase skin penetration and avoid the first-pass metabolism. This study aims to optimize the composition of phosphatidylcholine and Tween 80 in icariin-loaded transfersome vesicles to produce optimal physicochemical characteristics for transdermal applications. Method: Icariin transfersome was formulated using a thin-film hydration method with variations in phosphatidylcholine and Tween 80 concentrations. Optimization is performed using the Simplex Lattice Design (SLD) in Design Expert 13 software. The observed responses included particle size, polydispersity index (PDI), zeta potential, and Entrapment efficiency (EE%). The results of this study are the optimal formula of icariin transfersome, with a ratio of phosphatidylcholine and Tween 80 of 95%: 5%, resulting in ideal vesicle characteristics, namely a particle size of 106.15 nm, a zeta potential of -22.67 mV, a Polydispersity Index (PDI) of 0.37, and an Entrapment efficiency of 86.96%.

Keywords**:** Icariin; Transfersome; Phosphatidylcholine; Tween 80; Transdermal

INTRODUCTION

Icariin, the main flavonoid in *Epimedium* sp., has demonstrated phosphodiesterase type-5 inhibitory (PDE5I) activity and aphrodisiac effects in various in vivo studies. However, the only icariin formulations available on the market are in capsule form, a characteristic that results in low bioavailability due to poor water solubility and subsequently limits their clinical use. The development of icariin as an oral preparation faced pharmacokinetic constraints due to low water solubility (<100 µg/mL) and poor permeability (Log P = 0.81; pKa = 7.07), thereby lowering its bioavailability.[1,2,3]

One innovative approach to overcome this limitation is through flexible lipid vesicle-based transdermal delivery systems, such as transfersomes. Transfersome is a liposome-derived nanocarrier system formulated with surfactants as edge activators, so that it can penetrate the stratum corneum layer efficiently. The application of transfersome in topical dosage forms, particularly in gel preparations, has been reported to enhance drug penetration compared to conventional gel systems.[4]

Previous studies have explored Icariin formulations such as liposomes and SNEDDS to improve its solubility and oral bioavailability. However, these systems still face limitations in achieving sufficient skin penetration and stability during transdermal delivery. Transfersomes are distinguished from SNEDDS and conventional vesicles by their high deformability, enhanced skin penetration capacity, and superior stability.[5,6] Therefore, this study developed an optimized transfersome-based transdermal system, employing a Simplex Lattice Design to identify the optimal phospholipid–surfactant ratio. This optimization provides a novel insight into improving the entrapment efficiency and permeability of Icariin for potential application in erectile dysfunction therapy.[1,2,5–7] Statistical optimization using Design-Expert has also been widely applied in topical dosage form development to obtain optimal physicochemical characteristics of gel-based formulations.[8]

In this formulation, icariin is loaded into transfersome using a thin-layer hydration method. Phosphatidylcholine is a phospholipid that plays a critical role in the formation, stability, and performance of transfersomes. This lipid requires surfactants such as Tween 80 to stabilize the vesicle membrane, reduce particle size, enhance drug encapsulation, and establish the hydrophilic-lipophilic balance necessary for effective drug delivery. Therefore, the physicochemical characteristics of the transfersomes, namely particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency (EE%), were selected as optimization parameters.

Optimization was performed using the SLD method in Design-Expert® software version 13, with response parameters including particle size (<200 nm), PDI (<0.3), zeta potential, and EE% (>70%).[7] The SLD optimization is expected to show the potential influence of variations in phosphatidylcholine and Tween 80 on particle size, size distribution, and entrapment efficiency. Furthermore, the optimal phosphatidylcholine–Tween 80 ratio is expected to improve both the stability and the entrapment efficiency of the transfersomes. Therefore, this research contributes by defining critical formulation parameters, namely the component ratios required to produce transfersomes with appropriate physicochemical characteristics for the transdermal delivery of icariin.

METHODS

Equipment and Materials

The equipment used in this study consisted of a set of glassware, an analytical balance (Ohaus), a rotary evaporator (Haiqi instrument, China), a homogenizer (Biosfar, China), a digital autoclave (Hirayama, Japan), a particle size analyser, an ultracentrifuge (D3024, China), digital pH meter (Maskot phs-3c, China), magnetic stirrer, viscometer (Dj-8, China), sonicator bath (BK 2000, China), UV-Vis spectrophotometer (Rittun, China), micropipette (Secorex), and water bath (Maskot DWB GH).

The materials used were Icariin (Liftmode, USA), standard Icariin (Sigma-Aldrich 98%), analytical-grade ethanol (Alfa Kimia Semarang, Indonesia), phospholipon/soy Phosphatidylcholine obtained from (PT. Dwi Lab Bandung, Bandung, Indonesia), Tween 80 (Bratachem, Indonesia), and phosphate-buffered saline pH 7.4 ±0.2 (Biocomma).

Preparation procedure

Transfersome Icariin formulation

According to the formula, Icariin was dissolved in analytical-grade ethanol containing phospholipids and Tween 80, followed by homogenization. The mixture was evaporated using a rotary evaporator at 43 °C to form a thin lipid film on the flask wall, which was subsequently stored for 24 hours at 4 °C. Hydration was performed using PBS pH 7.4 to a volume of 10 mL with the aid of glass beads at 60 rpm for 1 hour. The resulting multilamellar vesicle dispersion was sonicated for 30 minutes in a bath sonicator at room temperature to yield a homogeneous transfersomal suspension. A statistical optimization approach was employed to determine the optimal formulation for icariin-loaded transfersomes. This involved setting constraints and goals for the independent variables (phosphatidylcholine and Tween 80 ratios) and the critical response parameters: particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency. The constraints and optimization criteria for the transfersome formulation are presented in Table 1.

Table 1. Phospholipid and surfactant composition constraints

Factor	Qty	Function
Phosphatidyl-choline	75-95	Phospho-lipid
Tween 80	5-25	Surfactant
Ethanol	15 ml	Solvent
PBS 7.4	Ad 100ml	Hydrating Medium

Determining Criteria for Each Response

This criterion establishes the desired goal or outcome of each response in formulation optimization. This response includes physicochemical parameters, and the stability of the resulting transfersomes is presented in Table 2.

Table 2. Determination of Criteria for Each Response

Response	Crite-ria	Unit	Range	Impor-tance
Particle Size	Mini-mum	Nm	50-200	+++++
Zeta Potential	Mini-mum	mV	(-20)-(-60)	+++
PdI	Mini mum	-	< 0.5	+++
Entrapment efficiency	Maxi-mum	%	70-95	+++++

Based on the response criteria established in Table 2, eight optimization formulas were designed using the SLD method. The composition of each formula is presented in Table 3.

Table 3. Transfersom Optimization Formula Design with *Design Expert 13 Simplex Lattice Design (SLD)*

Run	Formula		
	Icariin %	Phosphaticyclin (%) (A)	Tween 80 (%) (B)
1	1	75	25
2	1	90	10
3	1	80	20
4	1	95	5
5	1	85	15
6	1	85	15
7	1	75	25
8	1	95	5

Characterization of Transfersomes

Distribution of particle Size and Polydispersity Index.

The particle size and polydispersity index (PDI) of the icariin-loaded transfersome formulation were analyzed using a Particle Size Analyzer (PSA) with Aquadest as the dispersant. A total of 10 mL of the samples was placed in a cuvette for analysis. Based on previous studies, the target specifications of <200 nm for particle size and <0.5 for PDI are established benchmarks for optimizing skin penetration and formulation stability.[7]

Zeta Potential

Zeta potentials are used to predict the stability of the colloidal transfersome. Zeta potential measurements were performed on a Zeta Sizer. The suspension was diluted with analytical-grade water, and 10 mL of the diluted sample was transferred into a measurement cuvette, ensuring the absence of air bubbles. Zeta potential values between -20 and -30 indicate fairly good, and between -30 and -60 mV indicate good stability in the transdermal system.[7]

Entrapment Efficiency (%EE)

The levels of Icariin in the transfersome formulation were determined by a validated UV–Vis spectrophotometric method ($R^2 = 0.9982$; RSD = 0.53%; accuracy = 99.77%; LOD = 1.86 ppm; LOQ = 5.68 ppm), adapted from previously reported analytical procedures.[3,9,10] A total of 4 mL of suspension was centrifuged in an Amicon tube at 12,000 rpm for 1 hour. The filtrate was analyzed to determine the level of the free drug, at a wavelength of 268 nm, then the Entrapment efficiency was calculated using the formula:

$$\%\,EE = \left[\frac{total\ drug\ content - free\ drug\ content}{total\ drug\ contentt}\right] x100$$

Data Analysis and Optimization Procedure

Formulation optimization was conducted via a Simplex Lattice Design (SLD) using Design Expert version 13 (Stat-Ease Inc., USA). The independent variables were phosphatidylcholine concentration (75–95%) and Tween 80 concentration (5–25%), while the responses included particle size, polydispersity index (PDI), zeta potential, and entrapment efficiency (%EE). Each response was fitted into a quadratic polynomial model to evaluate the main and interaction effects of the independent variables. Model adequacy was verified using analysis of variance (ANOVA) by considering the p-value, R^2, adjusted R^2, and lack-of-fit.

The optimization predictions are presented as a 2D contour plot to visualize variable interactions. The optimum formula is selected based on the highest desirability value (0–1). Validation of the predicted optimum was performed by comparing the observed experimental data against the software-generated values using a one-sample t-test at a 5% significance level.

Morphology of Icariin Transfer Vesicle Shape

Morphological characterization of icariin transfersome vesicles was carried out using the Transmission Electron Microscopy (TEM) method. For TEM analysis, the transfersomal formulation was diluted, and a single drop was deposited onto a carbon-coated grid. The sample was allowed to adsorb onto the carbon film for 2 minutes at room temperature. The sample was allowed to adsorb onto the carbon film for 2 minutes at room temperature. After that, the sample is allowed to dry before analysis by TEM. This technique provides a detailed characterization of the vesicle internal structure, including size, shape, and lamellarity.[11]

RESULTS AND DISCUSSION

Icariin-loaded transfersomes prepared by the thin-layer hydration method exhibited a small particle size and homogeneous distribution. This method was chosen because it is relatively easy to do and does not take long. The formulation of transfersomes involves the systematic combination of phospholipids and surfactants in predetermined concentration ratios.

The formula optimization was carried out using Design-Expert 13 software with the SLD method. In this approach, two main components, namely phosphatidylcholine and Tween 80, were set as independent factors. Meanwhile, four response parameters—namely particle size, zeta potential, polydispersity index, and entrapment efficiency—were used as dependent variables. This approach aims to identify the optimal combination range of phosphatidylcholine and Tween 80.

The independent variables were constrained with phosphatidylcholine at 75–95% and Tween 80 at 5–25%, maintaining a total concentration of 100%. Based on these limits, the software. Based on these limits, the software then generated eight different formulation runs. The specific phosphatidylcholine-Tween 80 ratios for each experimental run are provided in Table 4.

The appearance of icariin transfersomes with various phosphatidylcholine and Tween 80 compositions is shown in Figure 1. Based on direct visual observation of the various icariin transfersome formulations (Figure 1), variations in opalescence were observed between formulations. This opalescent effect strongly suggests that nanoparticles have formed and are well dispersed within the system.

Figure 1. Visualization of Icariin transfersomes with different phosphatidylcholine and Tween 80 ratios.

The eight resulting formulas were then evaluated based on four predetermined response parameters. These four response parameters were chosen for their significant impact on key transfersome attributes. The evaluation results and the determination of the optimum area are presented in full in Table 4.

Before the optimization process, data analysis was performed on the experimental results listed in Table 4. The effect of each factor on the response was analyzed using ANOVA in Design-Expert 13 software. The results of this analysis are represented in mathematical model equations for each response and further visualized through response surface diagrams (Response Surface Methodology) in Figures 2 to 5. These graphs illustrate the changes in each factor relative to the variation in the response level, allowing for analysis of interactions between the independent variables studied.

1. Particle size test results

The optimization was carried out using the *Simplex Lattice Design (SLD)* method with two formulation components, Tween 80 (A) and phosphatidylcholine (B). The analysis showed that the quadratic model provided the best fit for the particle size response, with the following regression equation:

$$\text{Particle size (Y)} = 138.02A + 121.68B + 89.41(AB)$$

where A represents the Tween 80 concentration (%), B represents the phosphatidylcholine concentration (%), and AB represents their interaction effect. Both Tween 80 (A) and phosphatidylcholine (B) exhibited a positive contribution to particle size, and the interaction term (AB) also showed a significant positive effect, indicating that the simultaneous increase of both components tends to enlarge the vesicle size.

Table 4. The results of the measurement of particle size, zeta potential, index polydispersity, and %Entrapment efficiency.

Run	**Factor**	**Responds**				
	Lesitin	Tween 80	Size Particle (nM)	Zeta Potential (mV)	Polydisperssites Index (PDI)	% etrapment
1	75	25	132,77±0,49	-25,43±0,18	0,23±0,01	87,79±0,00
2	90	10	129,30±0,55	-26,32±0,20	0,19±0,02	83,05±0,00
3	80	20	146,87±0,96	-28,42±0,12	0,25±0,02	77,05±0,16
4	95	5	124,97±0,30	-29,89±0,19	0,33±0,02	87,76±0,08
5	85	15	148,63±0,47	-24,43±0,15	0,24±0,1	81,35±0,00
6	85	15	168,57±2,90	-23,75±0,22	0,22±0,02	73,79±0,10
7	75	25	143,07±0,40	-28,17±0,17	0,28±0,01	82,05±0,00
8	95	5	122,87±1,40	-26,71±0,22	0,36±0,02	84,52±0,00

Note: ζ = zeta potential; PDI = polydispersity index; EE = entrapment efficiency

The ANOVA results demonstrated that the interaction term (AB) was significant for particle size ($p = 0.0414$), while the linear effects were not significant ($p > 0.05$). The coefficient of determination ($R^2 = 0.6720$) and adjusted R^2 (0.5407) indicated that the model explained approximately 54% of the variation in the response, although the predicted R^2 (0.2813) suggested the possible presence of block effects or other sources of variability outside the model.

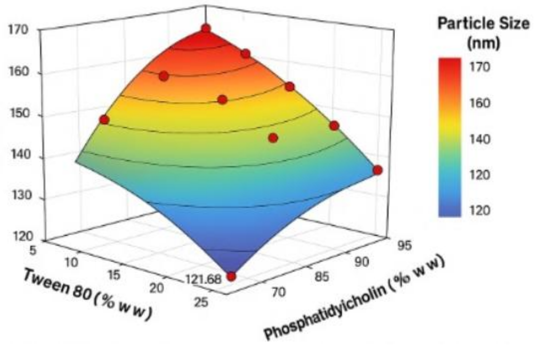

Figure 2. Surface *Response Graph* of Particle Size on icariin transfersome

Based on the *surface response* graph in figure 2, it can be seen that the variation in the concentration of Tween 80 and phosphatidylcholine has a significant influence on the size of Icariin transfersome particles. The smallest particle size (121,68 nm) was obtained at a combination of Tween 80 concentration of 5% and phosphatidylcholine of 95%, indicating a formulation optimization point. This change in particle size is closely related to the characteristics of each component. Tween 80, as a non-ionic surfactant, functions to lower the interface tension between the lipid and water phases, thus helping to form smaller and more stable vesicles. At sufficient concentrations, Tween 80 can coat the surface of the vesicles, prevent aggregation, and increase lipid solubility in aqueous media. However, at concentrations that are too high, Tween 80 can disrupt the integrity of phospholipid bilayers, causing increased membrane fluidity, which actually increases the particle size due to the structural instability of the vesicles.[11]

Meanwhile, phosphatidylcholine is the main component of the formation of bilayer vesicles, which are more rigid in nature. Increasing its concentration will increase the number of bilayer layers, so it tends to form larger vesicles. However, if the concentration is too high without being balanced with enough surfactants, the vesicle structure can become unstable and easily aggregate. Thus, the change in particle size on the graph reflects the complex interactions between surfactants and phospholipids. Optimal formulation is obtained when both components are in balance, where Tween 80 is sufficient to stabilize the vesicle structure without damaging the integrity of the membrane, and phosphatidylcholine is sufficient to form a compact bilayer structure. These results are in accordance with the literature that states that particle sizes below 200 nm are ideal for transdermal skin penetration.[12]

2. Zeta potential test results

The zeta potential analysis was performed using a mixture design with two main formulation components, Tween 80 (A) and phosphatidylcholine (B). The quadratic model obtained from the Design Expert analysis was expressed as follows:

$$\text{Zeta potential } (Y, \text{mV}) = -27.31A - 28.18B + 10.06(AB)$$

where A represents the concentration of Tween 80 (%), B represents the concentration of phosphatidylcholine (%), and AB indicates the interaction between Tween 80 and phosphatidylcholine. The negative coefficients of A and B suggest that an increase in either component individually tends to decrease the zeta potential value (making it more negative). However, the positive interaction term (AB) implies that at certain proportions, the combination of both components can increase the zeta potential (making it less negative). The predicted zeta potential value of −28.18 mV indicates moderate colloidal stability. This relatively high negative value signifies sufficient electrostatic repulsion between vesicles, preventing aggregation and maintaining dispersion stability.[13,14] Zeta potential is an important parameter in assessing the stability of nanoparticle systems, including transfersomes. Tween 80 and phosphatidylcholine influence the vesicle surface charge differently. Tween 80, being a non-ionic surfactant, can reduce interfacial tension and enhance vesicle hydration, which at certain concentrations may shift the zeta potential toward more positive values. However, at higher concentrations, Tween 80 can increase phospholipid solubility and disrupt the vesicle bilayer structure, leading to a more negative surface charge.

Generally, zeta potential values greater than +30 mV or lower than –30 mV indicate good electrostatic stability. Although the predicted value in this study (–28.18 mV) is slightly below this threshold, the presence of Tween 80 contributes additional steric stabilization, thereby enhancing the overall electrosteric stability of the Icariin transfersome system.

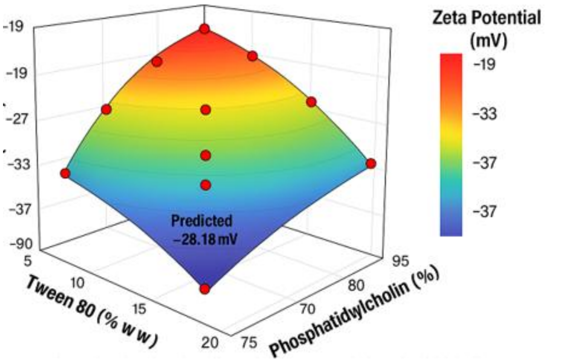

Figure 3. Response surface plot showing the effect of Tween 80 and phosphatidylcholine concentrations on the zeta potential of Icariin transfersomes.

Figure 3 shows that overall, the formulation with a high Tween 80 content and a low phosphatidylcholine content produced the most negative zeta potential, approaching the predicted value of -28.18 mV as seen in the response surface plot.

3. Index Polydispersity test results

The PdI values were analyzed during the optimization process using the SLD with two formulation components, Tween 80 (A) and phosphatidylcholine (B). The quadratic model obtained from the Design Expert analysis was expressed as follows:

$$\text{PdI (Y)} = 0.2665A + 0.3344B - 0.3332(AB)$$

where A represents the concentration of Tween 80 (%), B represents the concentration of phosphatidylcholine (%), and AB indicates the interaction between Tween 80 and phosphatidylcholine. The positive coefficients of A and B suggest that increasing either component individually tends to increase the PDI value, indicating a broader particle size distribution. Conversely, the negative coefficient of the interaction term (AB) indicates that the combination of both components can reduce PDI, resulting in a more homogeneous particle size distribution.

The ANOVA results revealed that the quadratic model was not statistically significant ($p = 0.0678$), although the AB interaction term approached significance ($p = 0.0503$). The coefficient of determination ($R^2 = 0.6592$) and adjusted R^2 (0.5229) indicated that the model could explain approximately 52.29% of the total variation in the data. The adequate precision value of 4.6718 (> 4) confirmed that the signal-to-noise ratio was sufficient for navigating the design space.

The predicted PdI value of 0.3344, observed at the optimum composition of Tween 80 (5%) and phosphatidylcholine (95%), falls within the acceptable range (< 0.5), indicating a narrow and homogeneous particle size distribution (15). These results suggest that the transfersome system achieved a desirable vesicle uniformity, which is beneficial for consistent drug diffusion and stability.

Based on the Response Surface graph for the Polydispersity Index (PdI) in figure 4, it can be seen that at the composition of Tween 80 = 5% and Phosphatidylcholine = 95%, the predicted PDI value is 0.33441. According to the literature, the PdI value considered ideal for a nanoparticle system is less than 0.5, which indicates a narrow and homogeneous particle size distribution.

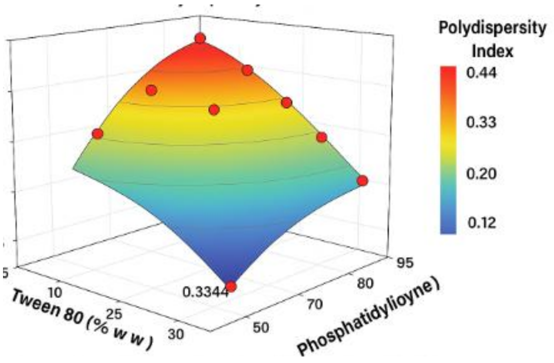

Figure 4. Response surface plot showing the effect of Tween 80 and phosphatidylcholine concentrations on the polydispersity index (PdI) of Icariin transfersomes.

However, in some cases, a PdI value between 0.3 and 0.4 is still acceptable if the formula remains in a stable state and does not undergo aggregation. The decrease in PDI at the optimal concentration of Tween 80 is due to the role of Tween 80 as an edge activator, which functions to soften phospholipid bilayers, increasing the flexibility and permeability of transfersome vesicles.[15] These surfactants are inserted into the bilayer structure and help form small, stable particles. However, if the concentration is too high, excessive missiles can be formed that damage the vesicle structure, thereby disrupting the stability and effectiveness of the system. Previous research has also mentioned that the balance between surfactants and lipids is essential for maintaining the stability of vesicles. In excess amounts, surfactants can actually disrupt the stability of the system, causing an increase in particle heterogeneity.[11,16]

4. Results of Entrapment Efficiency tests

The optimization of entrapment efficiency was conducted using the Simplex Lattice Design (SLD) with two main components, Tween 80 (A) and phosphatidylcholine (B). The quadratic model obtained from the Design Expert analysis was expressed as follows:

$$\text{Entrapment Efficiency (\%) (Y)} = 84.36A + 86.78B - 31.07(AB)$$

where A represents the concentration of Tween 80 (%), B represents the concentration of phosphatidylcholine (%), and AB indicates the interaction between the two components. The positive coefficients of A and B indicate that an increase in either Tween 80 or phosphatidylcholine concentration individually enhances the entrapment efficiency. However, the negative coefficient of the interaction term (AB) suggests that when both components are present in high concentrations, the entrapment efficiency tends to decrease due to possible competition between surfactant and lipid molecules within the vesicular structure.

The ANOVA results showed that the quadratic model was not statistically significant ($p = 0.0902$), although the interaction term (AB) had a significant effect ($p = 0.0402$). The coefficient of determination ($R^2 = 0.6180$) and adjusted R^2 (0.4652) indicated that the model could explain approximately 46.52% of the total variation in the data. The adequate precision value of 4.1130 (> 4) demonstrated that the signal-to-noise ratio was sufficient for exploring the design space.

The predicted entrapment efficiency value was 83.739%, indicating that Icariin was successfully incorporated into the transfersomal system with a high level of efficiency. These results are consistent with literature reports suggesting that phosphatidylcholine contributes to the formation of stable bilayer vesicles that can encapsulate lipophilic drugs efficiently, while Tween 80 enhances membrane fluidity and prevents vesicle aggregation.[11,14]

The formulation with the highest desirability value of 0.628 (Solutions 1) was selected as the optimal composition. This formulation was then used for validation by comparing the predicted and experimental results of key response parameters, including particle size, PDI, zeta potential, and entrapment efficiency. The validation confirmed that the experimental values were consistent with the model predictions, indicating the reliability of the optimization process and the robustness of the developed transfersomal system.

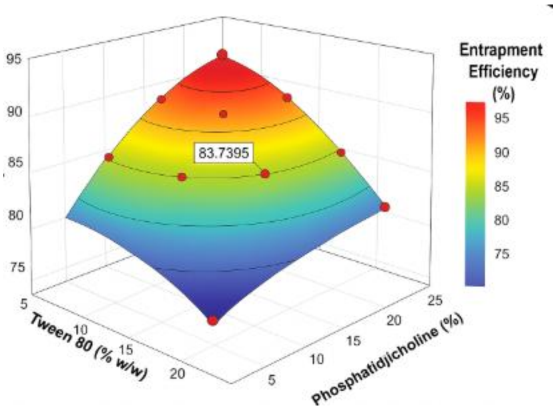

Figure 5. Response surface plot showing the effect of Tween 80 and phosphatidylcholine concentrations on the entrapment efficiency of Icariin transfersomes.

The surface response graph on Figure 5 shows a U-shaped trend, where Entrapment efficiency is higher at low (5%) and high (25%) Tween concentrations (25%) compared to medium concentrations (10–15%). This indicates that Tween 80 still has an effect on Entrapment efficiency, but the effect is non-linear. At low concentrations, Tween 80 acts as an edge activator that increases the fluidity of phospholipid bilayers, thereby facilitating the Entrapment of icariin into vesicles. Meanwhile, at medium concentrations, an increase in Tween 80 actually causes a disturbance of the bilayer structure which can reduce the ability of vesicles to absorb drugs. Interestingly, at high concentrations (25%), entrapment Efficiency increased again, possibly due to the formation of a more flexible vesicle structure or a combination of vesicles and miscellaneous that are still capable of trapping icariin. Previous research has stated that the selection and balance of edge activator concentrations greatly determine the deformability and entrapment capacity of the vesicular system. Therefore, the optimal formulation of 5% Tween 80, and 95% Phosphatidylcholine (F1) is considered to be the most suitable in maintaining the stability as well as efficiency of transfersome Entrapment.[11]

5. Determination of Optimum Formula

After the data were analyzed using the same software, the Design Mixture method identified one formulation as the optimal composition. The optimum formula can be determined based on the highest desirability value. Desirability is an optimization function that describes the extent to which a program meets the predetermined criteria for the final product. The closer the value is to 1.0, the better the program's ability to produce formulas that match the desired target.[17]

Between the two formulations proposed by Design-Expert 13, the combination of 95% phosphatidylcholine and 5% Tween 80, with a higher desirability value of 0.628, was selected as the optimal formulation. The resulting particle size of 121.7 nm falls within the optimal nanoscale range known to promote skin permeation. The resulting zeta potential of -28.177 mV indicates good dispersion stability, as a large negative value indicates a repulsive force between particles, thus preventing aggregation. The polydispersity index (PdI) of 0.334 is still within the acceptable range, indicating a fairly homogeneous particle size distribution. An entrapment efficiency of 83.4% demonstrates effective encapsulation of icariin in the formulated nanovesicles.

Following the optimization, the predicted optimal formulation was prepared and validated to verify the model's accuracy by comparing experimental results with predictions. Furthermore, the physical and chemical characteristics were verified based on responses such as particle size, PDI, zeta potential, and Entrapment efficiency. The validity of the optimization model was evaluated by comparing the experimental outcomes of the optimal formulation with Design-Expert predictions using statistical analysis.

The confirmed results showed an average particle size of 106.15 ± 2.33 nm, smaller than the predicted value of 121.68 nm ($p = 0.005$), indicating the potential for increased penetration and stability of the system. The value of the polydispersity index (PDI) of 0.374 ± 0.012 is within the acceptable homogeneity range. The potential zeta produced was –22.44 ± 0.279 mV, more positive than the predicted value (–28.18 mV) but remained within the colloidal stability limit (±20 mV) ($p = 0.000$), indicating that the system was quite electrostatically stable. The Entrapment efficiency of Icariin reached 86.96 ± 0.001%, higher than the predicted value (83.379%) with a significant difference ($p = 0.000$), indicating excellent entrapment ability. These results reinforce previous reports that transfersome formulations are able to improve the Entrapment efficiency and transdermal penetration of Icariin.[14]

Deformability is the ability of vesicles to undergo deformation without undergoing structural damage, which is very important in transdermal delivery systems such as transfersome. In this study, formulations with a concentration of Tween 80 of 5% showed the highest Entrapment efficiency, indicating that at this concentration the vesicles had optimal membrane flexibility. Tween 80 as an edge activator plays a role in disrupting the phospholipid bilayer structure in a controlled manner thereby increasing the fluidity and deformability of the vesicle membrane.

This allows the vesicles to penetrate deeper layers of the skin without breaking, while increasing the Entrapment capacity of the drug. Uncontrolled increased deformability, such as in excessively high concentrations of Tween 80, can actually lead to missile formation and decrease the stability and efficiency of Entrapment. Therefore, the selection and optimization of edge activator concentrations is key in achieving a balance between deformability, vesicle stability, and drug Entrapment ability in the transfersome system.[11,18]

The measurement results showed that the Icariin transfersome vesicles in this study were included in the *Large Unilamellar Vesicle* (LUV) category with a particle size range of <200 nm. This size meets the criteria of a transdermal nanoparticle delivery system because small particles have a better ability to pass through the stratum corneal layer effectively. The small particle size also allows for an increase in the surface area of contact with the skin and increases the likelihood of systemic absorption of the active substance. Therefore, these characteristics strongly support the successful transdermal delivery of Icariin as a therapy for erectile dysfunction.[18]

The polydispersity index (PdI) is used to show the particle size distribution in the system. The PdI values in this study were all <0.5, which indicates that the vesicles have a homogeneous size distribution. This value reflects a physically stable system, as the closer the value is to 0, the more uniform the particle size in the system becomes. Homogeneity of size is essential to maintain the stability and consistency of drug release from the delivery system.[19]

Based on the quadratic model generated by the Design Expert (SLD), both Tween 80 (A) and phosphatidylcholine (B) showed negative coefficients, indicating that an increase in either component tends to make the zeta potential more negative. This pattern can also be observed in the response surface plot (Figure 3), where the lowest zeta potential (−28.18 mV) was obtained at a combination of 5% Tween 80 and 95% phosphatidylcholine. These results are consistent with the theory that higher phosphatidylcholine content increases surface charge density, thereby enhancing electrostatic repulsion between vesicles and improving colloidal stability.

The negative surface charge mainly originates from the phosphatidylcholine component, which is zwitterionic and exhibits a net negative charge at the hydration medium pH (7.4). Tween 80, as a non-ionic surfactant, stabilizes the vesicles by steric hindrance rather than electrostatic effects, resulting in a moderately stable system with a zeta potential close to −30 mv.[14,20]

1. Morphology of Icariin Transfer Vesicle Form:

Morphological characterization of Icariin transfersome was performed using *Transmission Electron Microscopy* (TEM) to observe the shape, size, and surface structure of the vesicles in detail. TEM allows direct visualization of particles at the nanometer scale, so it can show the uniformity of the shape and distribution of the particles. The results of the observations showed that the transfersome vesicles had a spherical dominant morphology with clear membrane boundaries, indicating the success of the formulation process and the potential stability of the system. Documentation of the observation results of the morphology of Icariin transfersome *from TEM at 100,000x magnification (100 nm scale)* is presented in Figure 6.

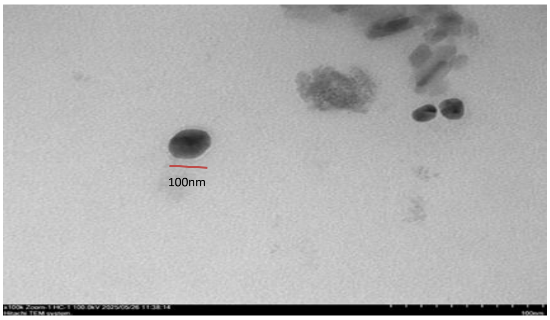

Figure 6. Morphology of icariin transfersome

The morphology of the formulated particles was observed using Transmission Electron Microscopy (TEM) and showed a predominantly spherical to oval particle shape with a clear edge boundary. The particle size observed visually ranges from 80–150 nm, according to the results of particle size analysis using PSA, which is 106.15 ± 2.33 nm. No large agglomerations or structural damage were found, indicating good morphology and adequate physical stability potential. The variation in the size of the visible particles is still within reasonable limits, given the characteristics of the nanoparticle system, which is polydispersity. The difference in observation methods between TEM (in dry) and PSA (in suspension) also explains the difference in values produced from the two techniques.[18]

CONCLUSION

The optimal transfersome formulation, with a ratio of phosphatidylcholine and Tween 80 of 95%: 5%, resulted in ideal vesicle characteristics, namely a particle size of 106.15 nm, a zeta potential of -22.67 mV, a Polydispersity Index (PDI) of 0.37, and an Entrapment efficiency of 86.96%.

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 any liability for claims relating to the content of this article will be borne by them.

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