

Transethosomal gels as carriers for the transdermal delivery of colchicine: statistical optimization, characterization, and ex vivo evaluation

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Introduction: Colchicine is used for the treatment of gout, pseudo-gout, familial Mediterranean fever, and many other illnesses. Its oral administration is associated with poor bioavailability and severe gastrointestinal side effects. The drug is also known to have a low therapeutic index. Thus to overcome these drawbacks, the transdermal delivery of colchicine was investigated using transethosomal gels as potential carriers.

Methods: Colchicine-loaded transethosomes (TEs) were prepared by the cold method and statistically optimized using three sets of 2⁴ factorial design experiments. The optimized formulations were incorporated into Carbopol 940® gel base. The prepared colchicine-loaded transethosomal gels were further characterized for vesicular size, dispersity, zeta potential, drug content, pH, viscosity, yield, rheological behavior, and ex vivo skin permeation through Sprague Dawley rats' back skin.

Results: The results showed that the colchicine-loaded TEs had aspherical irregular shape, nanometric size range, and high entrapment efficiency. All the formulated gels exhibited non-Newtonian plastic flow without thixotropy. Colchicine-loaded transethosomal gels were able to significantly enhance the skin permeation parameters of the drug in comparison to the non-ethosomal gel.

Conclusion: These findings suggested that the transethosomal gels are promising carriers for the transdermal delivery of colchicine, providing an alternative route for drug administration.

Keywords: transethosomes, ethosomal nanocarriers, colchicine, factorial design, skin permeation, rheology

Introduction

Colchicine is a potent alkaloid obtained from the dried corns and seeds of plants of the genus *Colchicum*, which belong to Liliaceae family. It has been used for the treatment of acute gout for >2,000 years. It is highly effective especially when given in the first 12–36 hours of the gouty attack.¹ Also, colchicine is the only available drug for treating familial Mediterranean fever (FMF).² It has been reported to improve height development and weight parameters in children with FMF.³ A daily lifelong administration of colchicine is required to prevent both the fever/pain attacks and the silent amyloid deposition.⁴ Although colchicine tablets have been prescribed in the United States since the 19th century, it was only in 2009, the US Food and Drug Administration (FDA) approved colchicine for the treatment of acute gout and FMF.⁵

Colchicine is known to have low therapeutic index^{1,6} and poor oral bioavailability (25%–50%) due to the extensive first-pass metabolism.⁷ In addition, the oral administration of colchicine is associated with mild to severe gastrointestinal side effects, including abdominal cramps and pain, nausea, vomiting, and diarrhea.⁸ These side

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effects commonly occur in around 80% of patients even at therapeutic doses.^{9,10} Alternatively, colchicine was administered intravenously, but this route of administration was associated with serious adverse effects such as tissue necrosis, cytopenias, disseminated intravascular coagulation, and even death.^{8,11} The intravenous administration was banned by the FDA in 2008.⁵ Thus, the use of colchicine has been limited due to all these drawbacks and adverse effects.

The transdermal delivery may offer a new route for the administration of colchicine to solve the problems of dose-dependent side effects and the poor bioavailability associated with its oral administration. Based on literature search, it has been found that only two studies have reported the transdermal delivery of colchicine using lipid-based vesicular systems. In both the studies, elastic liposomes have been used. In the first study, colchicine alone was encapsulated in these vesicles. The authors found that the vesicular size, entrapment efficiency (EE%), and the *in vitro* transdermal flux (J_{ss}) values through abdominal rat skin were 135 ± 11 nm, $66.3\% \pm 2.2\%$, and 44.4 ± 1.9 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively. In the second study, in an attempt to enhance the EE% and dermal deposition values and to prevent drug leakage upon storage, colchicine was complexed with β cyclodextrin, and the complex was then entrapped in elastic liposomes. Although, EE% was improved ($79.5\% \pm 1.6\%$), this was associated with a significant decrease in the flux (26.4 ± 1.2 $\mu\text{g}/\text{cm}^2/\text{h}$) and an increase in the vesicular size (152 ± 14 nm).^{12,13} Furthermore, no stability studies were reported for both the formulations. Stability study is important, because drug leakage from elastic liposomal vesicles commonly occurs during storage, especially when a hydrophilic drug like colchicine is incorporated.^{13–15}

Ethosomal nanocarriers are vesicular systems that contain a relatively high concentration of ethanol, in addition to phospholipids and water. They are specially designed for the dermal/transdermal delivery of therapeutic agents. Ethosomal systems are classified on the basis of their compositions into three classes: classical ethosomes, binary ethosomes, and transethosomes (TEs).¹⁶ TEs are the new generation of these nanocarriers; they were first developed in 2012.¹⁷ TEs contain the basic components of classical ethosomes and a penetration enhancer or an edge activator (surfactant) in their formula. The efficacy of TEs in enhancing the dermal and transdermal delivery of various therapeutic agents with different physicochemical properties has been proven by many researchers.^{17–20}

The aim of the present study was to investigate the potential use of transethosomal gels as carriers for the transdermal

delivery of colchicine as an alternative route for the drug administration. In this study, colchicine-loaded TEs were prepared and optimized statistically. The optimized formulations were incorporated into a gel base. Gels are the most suitable vehicles for the incorporation of ethosomal vesicles, because of their compatibility with these nanocarriers; and they provide the required viscosity and bioadhesive properties for the skin application. The prepared colchicine-loaded transethosomal gels were characterized in terms of vesicular size, dispersity (D), zeta potential (ZP), pH, drug content, and rheological properties. *Ex vivo* drug release studies were performed to determine and evaluate the skin permeation parameters. In addition, the stability of the optimized colchicine-loaded transethosomal gel was evaluated at different storage conditions.

Materials and methods

Materials

Colchicine (purity >97%) was purchased from Acros Organics (Morris, NJ, USA). Phospholipon 90G[®] (PL90G) (contains >90% phosphatidylcholine from soybean) was generously donated by Lipoid LLC (Newark, NJ, USA). Absolute ethanol (purity $\geq 99.5\%$) was bought from Merck KGaA (Darmstadt, Germany). Tween 20[®] (purity $\geq 99\%$), sodium stearate, and triethanolamine (purity $\geq 99\%$) were purchased from R & M Chemicals (Essex, UK). Sodium taurocholate, cholesterol, propyl paraben, methyl paraben, and Sephadex[®] G-25 were bought from Sigma-Aldrich Co., (St Louis, MO, USA). Propylene glycol (PG) and glacial acetic acid were obtained from QR[®]C (Selangor, Malaysia). Labrafil[®] M 1944 CS (Oleoyl macrogol-6 glycerides EP) was purchased from Gattefossé (Lyon, France). Carbopol 940[®] (cross-linked polyacrylate polymer) was a kind gift from Lubrizol Corporation (Wickliffe, OH, USA). Methanol and acetonitrile (high-performance liquid chromatography [HPLC] grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Ammonium acetate was bought from Bendosen Laboratory Chemicals (Bendosen, Norway). Ultrapure water was prepared by Elga purelab classic UVF water purification system. All the other organic solvents and chemicals used were either of analytical or HPLC grades.

Preparation of TEs

Three different formulas of TEs were prepared using the cold method.²¹ The organic phase was obtained by dissolving PL90G and surfactant (which is Tween 20[®], sodium taurocholate, or Labrafil[®]) in ethanol at 30°C. The aqueous phase (ultrapure water) was heated to 30°C and then added to the

organic phase dropwise with continuous stirring at 1,200 rpm using a magnetic stirrer. The mixing continued for 45 minutes to get the transethosomal dispersions that were further subjected to a size-reduction process by multiple extrusions through a 200 nm pore size membrane using Acrodisc® syringe filters (PALL Life Sciences, Port Washington, NY, USA).

Effects of formulation variables on the transethosomal properties

Three sets of 2⁴ factorial design experiments were applied to study the effects of formulation variables on the transethosomal properties. Three different types of surfactants, namely Tween 20®, sodium taurocholate, and Labrafil® were used in the study (Table 1). The full factorial design allows the quantification of the effects produced by the tested independent variables on the dependent variables such as the average vesicular size, *D*, and ZP and also shows the possible interactions between them.

The obtained results were analyzed using a Design-Expert® software, version 9.0.20 (Stat-Ease, Inc., Minneapolis, MN, USA). The significance of the individual independent variables and their possible interactions were evaluated using analysis of variance (ANOVA). Then, coded equations for identifying the relative impact of every factor were generated by comparing the coefficients of the factors using the software. Finally, 3D surface plots were used to visually explain the significant interactions between the independent variables and their effects on the studied responses.

Vesicular size, *D*, and ZP measurements

The mean vesicular size and *D* for various formulations were determined by photon correlation spectroscopy. The measurements were carried out using a Zetasizer 1000HSA (Malvern Instruments, Malvern, UK) at a fixed angle of 90° and a wavelength of 633 nm at 25°C±2°C. Before measurements, the

samples were diluted with distilled water to obtain a suitable scattering intensity within the range of 100–150 kilocounts per second. The zeta potential of the various formulations was measured using a Zetasizer nanoseries Nano-Z (Malvern Instruments). Before measurement, the samples were also diluted with distilled water.

Colchicine incorporation and optimization of EE%

TEs from all sets of designs with vesicular size ≤150 nm, *D* ≤0.3, and ZP ≥−20 mV were chosen for further study. The selected TEs were loaded with a starting concentration of 0.2%w/w of colchicine. The incorporation of colchicine was done by dissolving the drug in the aqueous phase during the preparation process of the ethosomal systems. The drug-loaded TEs were then characterized for the vesicular size, *D*, ZP, and EE%. Formulations that showed EE% of colchicine ≥75% were selected for further optimization steps of the EE%. Accordingly, the chosen formulations were further loaded with colchicine at concentrations of 0.3 and 0.5%w/w, respectively, and evaluated for the vesicular size, *D*, ZP, and EE%.

Separation of the free colchicine and determination of EE%

Sephadex G-25 minicolumn centrifugation technique was used to separate the colchicine-loaded TEs from the free (untrapped) colchicine.^{22,23} The minicolumn was prepared by weighing 1 g of the sephadex beads which were then allowed to swell overnight in 6 mL of distilled water at room temperature. Then, the supernatant was decanted, and the swollen suspension was degassed in an ultrasonic bath (Branson 5510; Emerson Electric Co., St Louis, MO, USA) for 10 minutes and subsequently poured into a pre-prepared 3 mL syringe plugged with glass wool. The minicolumn was packed by centrifugation at 1,000 rpm for 2 minutes by using a Centrifuge 5702R (Eppendorf, Hamburg, Germany). Colchicine-loaded TEs (1 mL) were placed on the top of the column and centrifuged at 1,000 rpm for 2 minutes. Eluent containing colchicine-loaded TEs was collected and the free colchicine was retained in the column by size exclusion chromatographic method. The procedure was repeated twice by adding 1 mL of distilled water on the top of the column each time to assist the elution of colchicine-loaded TEs. All the eluents were collected and kept for further analysis. Then, 100 µL of the eluent was dissolved in 5 mL of acetonitrile to lyse the vesicles and release the entrapped drug. The drug content was determined by a previously developed and validated

Table 1 Factorial designs for the preparation of transethosomes

	Values	
	Low	High
Independent variables		
A. Ethanol concentration	20%w/w	35%w/w
B. PL90G concentration	2%w/w	4%w/w
C. Surfactant concentration*	0.2%w/w	0.4%w/w
D. Number of extrusions	10 times	20 times
Dependent variables		
The average vesicular size (nm)		
Dispersity		
Zeta potential (mV)		

Note: *Three surfactants Tween 20®, sodium taurocholate, and Labrafil® were used separately in each factorial experiment.

Abbreviation: PL90G, Phospholipon 90G®.

HPLC method.²⁴ The analyses were carried out with a liquid chromatography device (Shimadzu, Kyoto, Japan). The separation was done using a Hypersil Gold C-18 column (250 mm × 4.6 mm ID × 5 μm; Thermo Fisher Scientific, Waltham, MA, USA), and the column was maintained at 30°C. The mobile phase consisted of acetonitrile:ammonium acetate buffer (20 mmol/L, pH = 4.85; 32:68 v/v). The flow rate was set at 1 mL/min while the sample injection volume was 10 μL. The detection wavelength was 353 nm.

The EE% was calculated using the following equation:

$$EE\% = \frac{\text{Weight of encapsulated colchicine}}{\text{Initial weight of colchicine}} \times 100 \quad (1)$$

Preparation of the colchicine-loaded transethosomal gels

Colchicine-loaded transethosomal gel was formed by incorporating the required amount of the optimized colchicine-loaded TEs in a pre-prepared gel base at the ratio of 4:1 with gentle stirring, followed by manual mixing for 10 minutes. The gel base was prepared by dispersing Carbopol 940® (2%w/w) in distilled water and leaving to swell overnight in the refrigerator. Subsequently, methyl paraben and propyl paraben were dissolved in PG and then added to the gel mixture. The gel was then neutralized by the dropwise addition of triethanolamine with continuous manual mixing until pH value of 7.0 was reached, to get the final homogeneous transparent gel base. The composition of the gel base is summarized in Table 2. The non-ethosomal (NE) colchicine gel was prepared by dissolving colchicine in hydroethanolic solution (35%w/w) and then mixing with the gel base using the same ratio and procedure. The final colchicine concentration in all the prepared gels was 0.24%w/w.

Characterization of the colchicine-loaded transethosomal gels

Determination of pH

The pH of various gels was determined using a digital pH meter (HI-2213 bench pH Meter; Hanna Instruments,

Woonsocket, RI, USA). Approximately 0.5 g of various transethosomal gels was dispersed in 20 mL of distilled water and stirred for 30 minutes using a magnetic stirrer at room temperature; the pH sensor probe electrode was then immersed into the dispersed gel, and the pH of the formulation was read from the digital display.

Vesicular size, \bar{D} , and ZP of the incorporated TEs

Mean size, \bar{D} , and ZP were measured for the colchicine-loaded transethosomal vesicles after their incorporation in the gel base. Colchicine-loaded gels were dispersed in 20 mL of distilled water and stirred for 30 minutes using a magnetic stirrer at room temperature. The samples were then diluted with distilled water, and the mean vesicular size, \bar{D} , and ZP values were measured according to the methods described above.

Rheological behavior, viscosity, and yield measurements

The rheological properties of the colchicine-loaded transethosomal gels were evaluated using a Viscotech® rheometer (Rheologica Instruments AB, Lund, Sweden) equipped with a cone (C40-4) and plate measuring system. About 1 g of the gel sample was carefully placed on the plate to ensure that a correct gap-fill was obtained. The gap distance was set at 1 mm. After bringing down the cone, the sample was left to equilibrate at 25°C for 25 seconds. A shear stress for the ascending flow curve was applied at 1–200 Pa while for the descending flow curve it was at 200–1 Pa in a linear pattern, and the rate measurements were recorded. A fresh gel sample was used for each run. The shear rate versus shear stress data were fitted to rheological models. The gel formulations showed non-Newtonian plastic flow and fitted the Bingham model of rheological behavior. Accordingly, the rheological parameters were calculated based on the following equation:

$$\log G = N \log (S - F) - \log \eta \quad (2)$$

where G is the shear rate in second^{-1} , S is the shear stress in Pascal, F is the yield value, η is the viscosity, and N is the slope of $\log (S - F)$ against $\log G$ plot. When N is 1, plastic flow with the Bingham model is indicated.²⁵

Ex vivo skin permeation studies

The ex vivo skin permeation studies were carried out using the skin of male Sprague Dawley rats at the age of

Table 2 Composition of the gel base

Ingredients	Concentration
Carbopol 940®	2%w/w
Methyl paraben	0.02%w/w
Propyl paraben	0.01%w/w
Propylene glycol	1%w/w
Distilled water	Up to 100%w/w

6–7 weeks and weighing ~180–200 g. The study was conducted according to the Universiti Sains Malaysia guidelines for the care and use of animals for scientific purposes. The experimental protocol was approved by the Animal Ethics Committee of Universiti Sains Malaysia (USM/Animal Ethics Approval/2014 (91) (572)). The rats were obtained from the animal house of the university.

Preparation of the skin

Rats were sacrificed using an overdose of carbon dioxide (CO₂) gas. The hair on the dorsal side of the animal was carefully removed using an electrical hair clipper. The shaven part of the skin (full thickness) was excised from the animal, and the subcutaneous fat tissue was carefully removed. Subsequently, the excised skin was washed thoroughly with phosphate-buffered saline (PBS, pH=6.4) and examined visually for integrity or any cracks or furrows, and then sliced into sections and used for permeation studies.

Skin permeation studies

The ex vivo skin permeation experiments were carried out to determine and compare the skin permeation parameters between the optimized transethosomal gels and the NE gel formula. All the gels contained the same concentration of colchicine (0.24%w/w). The experiments were performed using standard 9 mm jacketed clear glass static Franz diffusion cells (V3 3-Station vertical cell stirrer; PermeGear Inc., Hellertown, PA, USA). The Franz diffusion cell was composed of two compartments; the donor and the receptor compartments. The effective diffusion area was 0.636 cm² and the volume of the receptor compartment was 5 mL. Prior to permeation experiments, a piece of back skin of rat was mounted between the donor and the receptor compartments while keeping the stratum corneum side of the skin facing toward the donor compartment. The receptor compartment was filled with PBS (pH =6.4), stirred at 500 rpm, and the temperature was maintained at 37.0°C±0.1°C. After equilibration for 30 minutes, 1 g of each gel formulation was placed in the donor compartment. At predetermined time intervals of 1, 2, 4, 6, 8, 12, and 24 hours, a volume of 0.5 mL was withdrawn from the receptor nozzle and immediately replaced with an equal volume of fresh medium (at the same temperature) to maintain the receptor phase volume at a constant level. The amounts of colchicine in the collected samples were quantified using the HPLC method described above.

Data analysis of the skin permeation studies

The cumulative amount of colchicine permeated through the rat skin (Q_t) into the receptor medium was calculated using the following equation:

$$Q_t = \left[C_t V_r + \sum_{i=1}^{t-1} C_i V_s \right] \quad (3)$$

where C_t is the concentration of colchicine (µg/mL) at each sampling interval, V_r is the receptor volume of the individual Franz diffusion cell (5 mL), C_i is the concentration of colchicine (µg/mL) at the *i*th sample, and V_s is the sampling volume (0.5 mL). The data were expressed as the cumulative amount of colchicine per unit area of the rat skin, Q_t/A (A=0.636 cm²).²⁶

For the calculation of the permeation flux (J_{ss}), the cumulative amount of colchicine from various colchicine-loaded gels permeated per unit area through the rat skin was plotted against time (t). The slope and intercept of the linear portion of the plots were derived by regression, and the flux was calculated as the slope divided by the skin surface area (Equation 4).

$$J_{ss} = (dQ/dt)/A \quad (4)$$

where J_{ss} is the steady state flux (µg/cm²/h), A is the effective diffusion area of the skin through which the drug permeation takes place, and dQ/dt is the amount of drug passing through the skin per unit time at a steady-state (µg/h).²⁷

The permeability coefficient (Kp, measured in cm/h) was calculated from the following equation:

$$Kp = J_{ss}/Cd \quad (5)$$

where Cd represents the drug concentration applied on the skin in the donor compartment.²⁸

Determination of the drug content in the optimized transethosomal gel

The drug content in the optimized colchicine-loaded transethosomal gel was determined by weighing 0.5 g of the formula and then dispersing it in 20 mL of distilled water under continuous mixing with a magnetic stirrer for 60 minutes. The dispersion was then sonicated in an ultrasonic bath (Branson 5510) for 10 minutes. A suitable volume was removed and diluted with the mobile phase, and the drug content was measured using HPLC.

Transmission electron microscopy (TEM)

TEM was used to study the morphology and for further verification of the vesicular size of the optimized colchicine-loaded transethosomal formulation in its initial suspension form and in the gel formulation. The samples were visualized under a TEM (LIBRA 120; Carl Zeiss Meditec AG, Jena, Germany). For the colchicine-loaded transethosomal suspension, a fixed volume of the formulation was diluted 100-fold with distilled water, while for the colchicine-loaded transethosomal gel, 0.5 g of the gel was dispersed in 25 mL of distilled water and stirred for 60 minutes using a magnetic stirrer. A drop of either dispersion was placed onto a carbon-coated copper grid, air dried, and then negatively stained with 2% phosphotungstic acid. The grid was allowed to dry in the air and then viewed under the TEM.

Stability studies

The stability studies of the final optimized colchicine-loaded transethosomal gel formulation were carried out by placing freshly prepared samples of the gel in two different storage conditions; namely refrigerated at $4^{\circ}\text{C}\pm 2^{\circ}\text{C}$ and room temperature at $25^{\circ}\text{C}\pm 2^{\circ}\text{C}/60\%\pm 5\%$ relative humidity (RH) for 3 months. The prepared formulations were evaluated in terms of vesicular size, D , ZP, pH, drug content, viscosity, yield, and rheological behavior.

Statistical analyses

All experiments were repeated at least three times. The results obtained from the characterization, skin permeation, and stability studies of the various colchicine-loaded formulations were analysed statistically using one-way ANOVA and post hoc Tukey–honestly significant difference tests. All the statistical analyses were performed using a Minitab® statistical software, version 17.2.1.0 (Minitab, Inc., State College, PA, USA). Data are expressed as mean values with the standard deviation (mean \pm SD), and a P -value < 0.05 was considered statistically significant.

Results and discussion

Preparation and optimization of TEs

The cold method was adopted for the preparation of TEs as it is simple and does not require highly specialized equipment or extreme conditions. Vesicular size is an important parameter that should be considered in the preparation of nanocarriers for transdermal drug delivery. It has been reported that the vesicular size should be < 200 nm to be suitable for this route of administration.^{29,30} Accordingly, the prepared vesicles were subjected to multiple extrusions through 200 nm pore

size membrane, to produce smaller vesicular size. In the initial stage of the preparation, it was found that the vesicular size and D of all the prepared ethosomal systems before the multiple extrusion process were > 340 nm and > 0.2 , respectively. It was recommended in the literature that the number of extrusions should be either 10 or 20 times to get the required homogeneous dispersions.^{31–33} In order to study the effects of the number of extrusions on the vesicular properties, both the numbers of extrusions (ie, 10 and 20 times) were selected as the low and high levels and studied for all the formulations.

Ethanol is a major constituent of the ethosomal systems, giving them their unique characteristics. It is usually used at concentrations of 10%–50%. However, at high ethanol concentrations and beyond certain levels, it may cause the vesicular bilayers of TEs to be leaky due to the partial solubilization of the lipid bilayers, which may lead to a significant decrease in EE%. Further increase in its concentration would lead to vesicle solubilization. Therefore, in the preliminary studies, ethanol concentration at higher levels (30, 35, 40, and 45%w/w) was investigated. It was found that ethanol concentrations of 40%w/w caused the vesicular size to increase. This could be because the vesicular bilayers were partially solubilized (ie, sublysis stage). At higher ethanol concentration of 45%w/w, a complete solubilization of the vesicles occurred, and the TEs no longer existed. Therefore, the highest level of ethanol concentration used in this study was fixed at 35%w/w. The optimized lower level of ethanol concentration was selected at 20%w/w, as it was found that at lower levels of ethanol concentrations (below 20%w/w) the vesicular size was increased to more than the required limit of 200 nm. This finding is in agreement with previous studies which showed that the vesicular size increased by decreasing ethanol concentration.^{21,34}

The commonly used phospholipid concentration in the preparation of ethosomal systems ranged between 0.5 and 5%w/w.³⁵ In this study, the selected upper and lower levels of the phospholipid (PL90G) concentrations were 4 and 2%w/w, respectively. It was observed that at concentrations $> 4\%$ w/w, the size of the vesicles was > 200 nm. Similar results for the size increment were obtained at concentrations $< 2\%$ w/w, as the vesicle bilayers reach the sublysis concentration due to the presence of a relatively low concentration of lipid and very high concentration of ethanol. The vesicles at this stage become leaky, and hence lower EE% is expected.

In the preliminary stage of the study, various types and concentrations (0.05%–0.5%w/w) of surfactants such as Tween 20®, Labrafil® (nonionic surfactants), sodium taurocholate, sodium stearate (anionic surfactants), or vesicle

stabilizers such as cholesterol were investigated. Tween 20®, Labrafil®, and sodium taurocholate were found to produce homogeneous and stable vesicular dispersions within the concentration range of 0.2%–0.4%w/w. In contrast, sodium stearate and cholesterol led to the formation of nonhomogeneous and unstable dispersions, and they were excluded from the study.

The composition of all formulations and the results of the studied responses of all the factorial design experiments are shown in Tables 3–5. Formulations with vesicular size ≤ 150 nm, $D \leq 0.3$, and $ZP \geq -20$ mV were bolded in the tables. These TEs were selected for further studies.

Analysis of the full factorial designs

The results of regression analysis of the size, D , and ZP responses of all the designs are summarized in Table 6. It was revealed that the produced models for the vesicular size, D , and ZP are significant ($p < 0.05$) with nonsignificant lack of fit for all the three conducted factorial experiments. To confirm that the proposed models can be used to navigate the design space, “adequate precision” values were calculated and were found to be >4 (the desirable value) in all responses and for all the factorial designs, indicating adequate signal to noise ratio. The predicted R^2 values were in a reasonable agreement with the adjusted R^2 values in all responses, except for the D in transethosomes with sodium taurocholate (TENa) design,

Table 3 Experimental design and value of each factor and responses for transethosomes with Tween 20® (TET) design

Code	Factors				Responses		
	A	B	C	D	Size (nm)	D	ZP (mV)
TET 1	20	2	0.2	10	145.6±3.5	0.164±0.013	-25.97±1.65
TET 2	35	2	0.2	10	110.4±5.9	0.167±0.043	-26.13±0.40
TET 3	20	4	0.2	10	152.0±3.2	0.124±0.040	-19.93±0.60
TET 4	35	4	0.2	10	107.8±5.5	0.143±0.023	-20.93±0.82
TET 5	20	2	0.4	10	129.6±2.3	0.090±0.033	-18.57±2.98
TET 6	35	2	0.4	10	108.5±10.2	0.120±0.022	-26.63±0.74
TET 7	20	4	0.4	10	133.7±12.7	0.112±0.024	-17.50±1.88
TET 8	35	4	0.4	10	89.5±3.2	0.145±0.025	-22.00±0.94
TET 9	20	2	0.2	20	142.4±1.8	0.154±0.007	-25.67±1.60
TET 10	35	2	0.2	20	103.6±3.4	0.119±0.012	-22.30±1.50
TET 11	20	4	0.2	20	146.3±3.7	0.113±0.010	-18.03±1.02
TET 12	35	4	0.2	20	99.6±4.2	0.138±0.041	-19.03±1.58
TET 13	20	2	0.4	20	125.2±2.3	0.085±0.023	-19.97±0.98
TET 14	35	2	0.4	20	109.5±11.9	0.086±0.036	-23.40±1.15
TET 15	20	4	0.4	20	127.7±8.6	0.080±0.011	-17.40±1.34
TET 16	35	4	0.4	20	86.0±3.4	0.150±0.028	-19.10±1.85

Notes: Each value is expressed as mean \pm standard deviation ($n=3$). A, ethanol concentration (%w/w); B, PL90G concentration (%w/w); C, Tween 20® concentration (%w/w); D, number of extrusions. Formulations with vesicular size ≤ 150 nm, $D \leq 0.3$, and $ZP \geq -20$ mV are shown in bold.

Abbreviations: D , dispersity; ZP , zeta potential; PL90G, Phospholipon 90G®.

Table 4 Experimental design and value of each factor and responses for transethosomes with sodium taurocholate (TENa) design

Code	Factors				Responses		
	A	B	C	D	Size (nm)	D	ZP (mV)
TENa 1	20	2	0.2	10	166.8±1.0	0.160±0.014	-24.87±2.39
TENa 2	35	2	0.2	10	94.3±3.4	0.131±0.013	-31.30±3.90
TENa 3	20	4	0.2	10	146.4±2.8	0.092±0.041	-26.37±1.72
TENa 4	35	4	0.2	10	90.6±3.1	0.085±0.005	-26.77±1.01
TENa 5	20	2	0.4	10	159.3±7.5	0.129±0.017	-31.17±1.93
TENa 6	35	2	0.4	10	97.4±3.6	0.108±0.020	-37.00±0.93
TENa 7	20	4	0.4	10	127.3±2.7	0.110±0.023	-25.40±0.88
TENa 8	35	4	0.4	10	94.2±2.1	0.154±0.015	-33.47±0.52
TENa 9	20	2	0.2	20	158.0±8.7	0.093±0.034	-21.47±2.11
TENa 10	35	2	0.2	20	85.8±1.9	0.115±0.015	-24.10±0.16
TENa 11	20	4	0.2	20	140.0±3.6	0.110±0.004	-22.63±0.42
TENa 12	35	4	0.2	20	85.4±5.4	0.087±0.007	-25.47±0.98
TENa 13	20	2	0.4	20	152.6±9.2	0.098±0.033	-30.30±0.54
TENa 14	35	2	0.4	20	92.4±3.8	0.111±0.019	-31.17±2.21
TENa 15	20	4	0.4	20	128.8±3.3	0.130±0.041	-26.77±0.29
TENa 16	35	4	0.4	20	88.9±3.9	0.117±0.052	-32.10±1.53

Notes: Each value is expressed as mean \pm standard deviation ($n=3$). A, ethanol concentration (%w/w); B, PL90G concentration (%w/w); C, sodium taurocholate concentration (%w/w); D, number of extrusions. Formulations with vesicular size ≤ 150 nm, $D \leq 0.3$, and $ZP \geq -20$ mV are shown in bold.

Abbreviations: D , dispersity; ZP , zeta potential; PL90G, Phospholipon 90G®.

which had a negative predicted R^2 value implying that the overall mean is a better predictor of the response.³⁶

Coded equations (Table 7) for the studied responses (ie, vesicular size, D , and ZP) were generated by the software

Table 5 Experimental design and value of each factor and responses for transethosomes with Labrafil® (TEL) design

Code	Factors				Responses		
	A	B	C	D	Size (nm)	D	ZP (mV)
TEL 1	20	2	0.2	10	168.7±3.9	0.113±0.008	-25.60±3.48
TEL 2	35	2	0.2	10	111.5±1.5	0.197±0.006	-22.57±1.65
TEL 3	20	4	0.2	10	159.1±1.5	0.183±0.011	-17.37±1.20
TEL 4	35	4	0.2	10	114.3±2.6	0.262±0.021	-25.17±8.09
TEL 5	20	2	0.4	10	168.2±2.9	0.178±0.019	-20.90±1.16
TEL 6	35	2	0.4	10	169.9±4.2	0.230±0.004	-20.30±4.17
TEL 7	20	4	0.4	10	159.2±6.8	0.152±0.018	-24.40±7.30
TEL 8	35	4	0.4	10	101.1±2.0	0.194±0.009	-20.07±0.57
TEL 9	20	2	0.2	20	167.1±1.1	0.132±0.024	-23.80±1.02
TEL 10	35	2	0.2	20	94.5±2.9	0.168±0.027	-18.53±0.92
TEL 11	20	4	0.2	20	159.1±0.6	0.179±0.013	-18.03±1.07
TEL 12	35	4	0.2	20	109.3±4.8	0.220±0.030	-18.30±1.73
TEL 13	20	2	0.4	20	162.3±5.5	0.180±0.015	-22.90±2.82
TEL 14	35	2	0.4	20	133.1±2.7	0.150±0.008	-10.58±3.44
TEL 15	20	4	0.4	20	149.2±1.7	0.130±0.021	-16.13±4.57
TEL 16	35	4	0.4	20	104.0±3.6	0.154±0.033	-12.93±1.92

Notes: Each value is expressed as mean \pm standard deviation ($n=3$). A, ethanol concentration (%w/w); B, PL90G concentration (%w/w); C, Labrafil® concentration (%w/w); D, number of extrusions. Formulations with vesicular size ≤ 150 nm, $D \leq 0.3$, and $ZP \geq -20$ mV are shown in bold.

Abbreviations: D , dispersity; ZP , zeta potential; PL90G, Phospholipon 90G®.

Table 6 Results of regression analysis for the responses of the size, \bar{D} , and ZP for all the designs

Design	Responses	R ²	Adjusted R ²	Predicted R ²	Adequate precision	Significant factors
TET	Size (nm)	0.9051	0.8856	0.8562	21.096	A, C, D, AB, AC, BC
	\bar{D}	0.4695	0.3766	0.236	7.007	A, C, AB, BC
	ZP (mV)	0.8068	0.7611	0.6918	11.683	A, B, C, D, AC, AD, BC, ABC
TENa	Size (nm)	0.9721	0.9672	0.9598	35.389	A, B, C, D, AB, AC
	\bar{D}	0.4258	0.2504	-0.0207	5.79	BC, ABD
	ZP (mV)	0.861	0.8079	0.723	14.922	A, B, C, D, BC, BD, ABC
TEL	Size (nm)	0.9847	0.9776	0.9656	30.928	A, B, C, D, AB, AC, AD, BC, BD, CD, ABC, ABD, BCD, ABCD
	\bar{D}	0.7858	0.7204	0.6192	12.784	A, B, D, AC, AD, BC
	ZP (mV)	0.5751	0.376	0.0441	5.942	A, C, D, AB

Note: A, B, C, and D refer to the independent variables selected for every factorial design experiment as illustrated in Table 1.

Abbreviations: \bar{D} , dispersity; ZP, zeta potential; TET, transethosomes with Tween 20[®]; TENa, transethosomes with sodium taurocholate; TEL, transethosomes with Labrafil[®].

to determine the magnitude of the effect of individual factors and the interactions between factors in all the designs. A positive value before a factor in the response equation denotes a positive impact (synergistic effect) that favors optimization, while a negative value indicates a negative impact (antagonistic effect) or inverse relation between the factor and the response.³⁷ The 3D response surface plots are useful to understand the effect of interactions between the factors on single response (Figures 1–3). Only the statistically significant interactions ($p < 0.05$) were selected for these plots.

Effects of formulation variables on the transethosomal properties

For ease of understanding, the effects of individual factors on the vesicular size, \bar{D} , and ZP of the prepared ethosomal systems in all the designs are summarized in Table 8.

Factors that had statistically significant effects ($p < 0.05$) on the responses are labeled with asterisks.

Effects of formulation variables on the vesicular size and \bar{D}

The vesicular size of ethosomal systems plays an important role in skin permeation. Smaller vesicular size (< 200 nm) facilitates the TEs to pass through the small pores of the skin leading to more enhanced skin permeation. The size of all the prepared TEs was < 200 nm (ranging from 85.4 to 169.9 nm) with low \bar{D} values (ranging from 0.08 to 0.262) as illustrated in Tables 3–5. \bar{D} values < 0.3 are considered ideal and indicate a narrow size distribution.³⁸

It was observed that increasing ethanol concentration significantly reduced the vesicular size, but within the concentration range of 20%–35%w/w ($p < 0.05$), as shown in 3D surface plots (Figures 1–3). Increasing ethanol concentration beyond

Table 7 Coded equations of the size, \bar{D} , and ZP for all the designs

Design	Equations for vesicular size	Equations for \bar{D}	Equations for ZP
TET	Size = +119.84 – 17.97A – 2.01B – 6.12C – 2.28D – 4.12AB + 2.64AC – 2.49BC – 2.00ABC	\bar{D} = +0.12 + 9.283E–003A + 1.294E–003B – 0.016C – 8.627E–003D + 9.323E–003AB + 7.701E–003AC + 0.012BC	ZP = –21.41 – 1.03A + 2.17B + 0.84C + 0.80D + 6.250E–003AB – 1.18AC + 0.69AD – 0.60BC + 0.66ABC
TENa	Size = +119.26 – 28.15A – 6.56B – 1.64C – 2.77D + 5.21AB + 3.74AC – 1.24BC	\bar{D} = +0.11 – 8.569E–004A – 3.665E–003B + 5.121E–003C – 6.806E–003D + 1.065E–003AB + 8.361E–004AD + 0.012BC + 7.122E–003BD + 1.142E–003CD – 9.853E–003ABD – 5.864E–003BCD	ZP = –28.15 – 2.02A + 0.78B – 2.77C + 1.40D – 0.054AB – 0.49AC + 0.57AD + 0.71BC – 0.77BD – 0.56CD – 0.78ABC – 0.53ABD + 0.40ACD
TEL	Size = +139.40 – 22.20A – 7.50B + 3.96C – 4.60D – 2.53AB + 5.85AC – 2.40AD – 7.49BC + 3.07BD – 1.62CD – 6.93ABC + 3.40ABD + 0.16ACD + 1.36BCD + 2.10ABCD	\bar{D} = +0.18 + 0.021A + 7.936E–003B – 5.342E–003C – 0.012D + 2.633E–003AB – 9.533E–003AC – 0.012AD – 0.021BC – 1.199E–003BD – 5.365E–003CD + 4.768E–003ABD	ZP = –19.85 + 1.29A + 0.80B + 1.32C + 2.20D – 1.36AB + 1.26AC + 1.27AD – 0.66BC + 0.50BD + 0.69CD + 0.69ABC – 0.47ABD + 0.051ACD + 0.46BCD – 1.13ABCD

Note: A, B, C, and D refer to the independent variables selected for every factorial design experiment as illustrated in Table 1.

Abbreviations: \bar{D} , dispersity; ZP, zeta potential; TET, transethosomes with Tween 20[®]; TENa, transethosomes with sodium taurocholate; TEL, transethosomes with Labrafil[®].

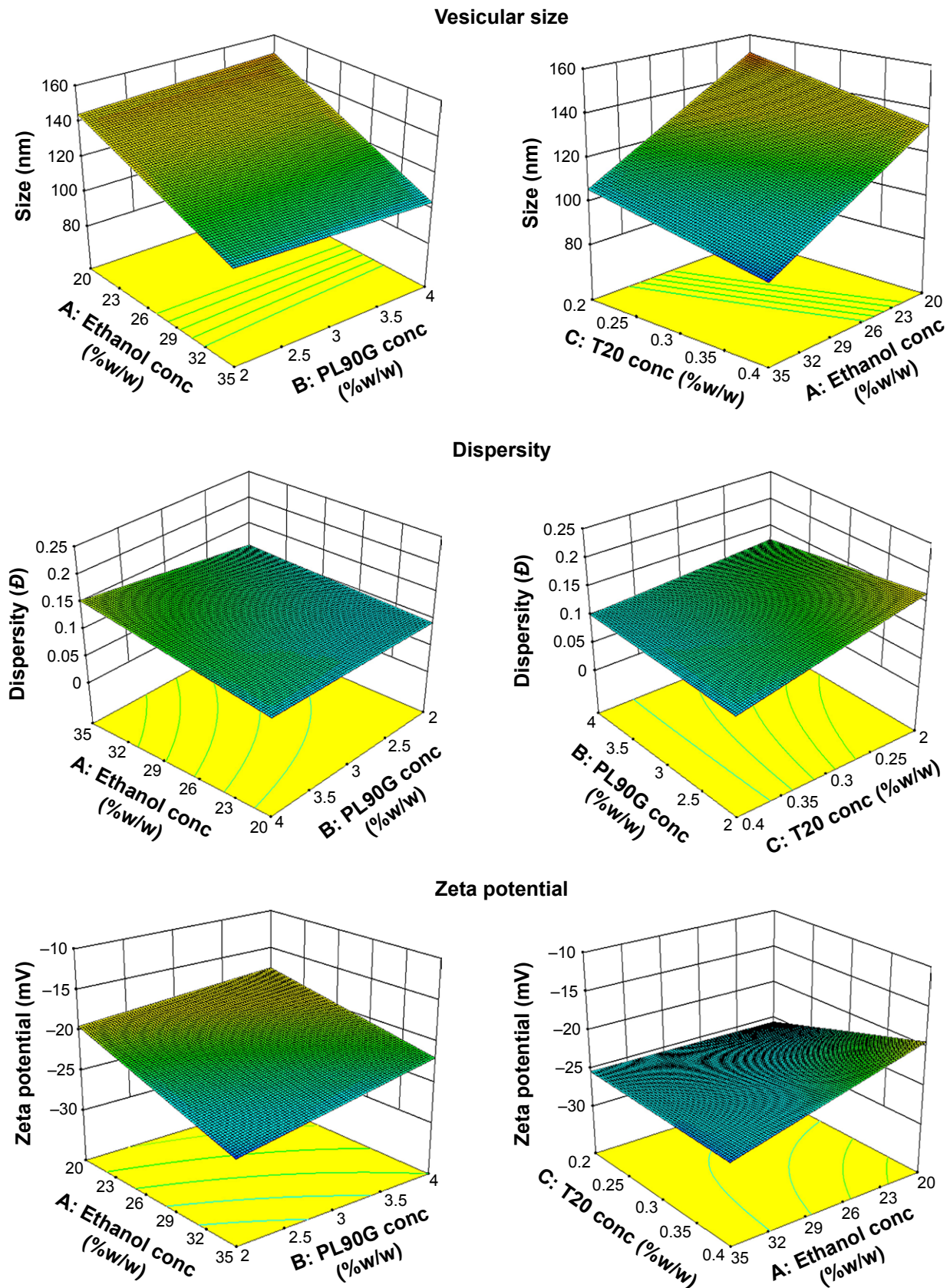
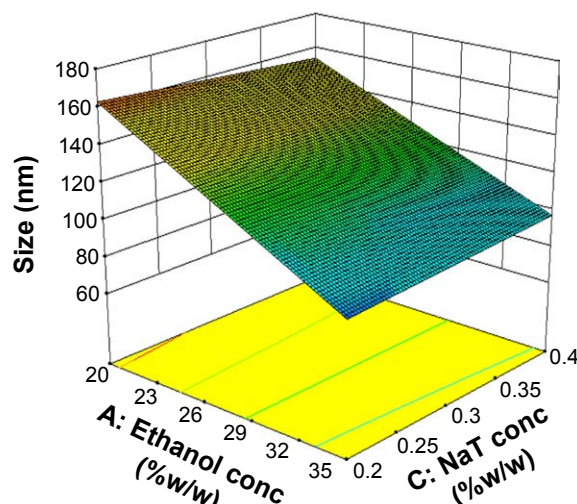
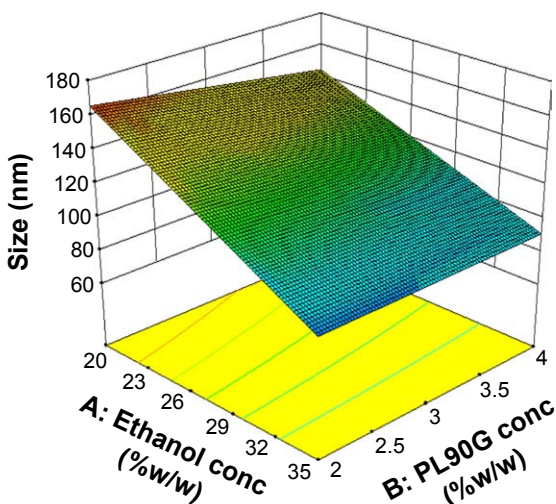
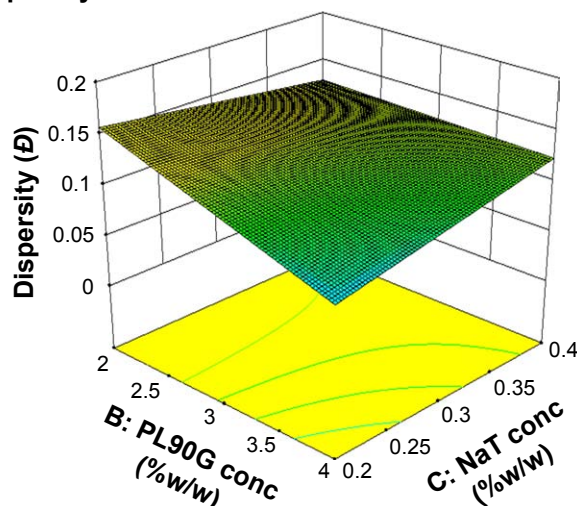
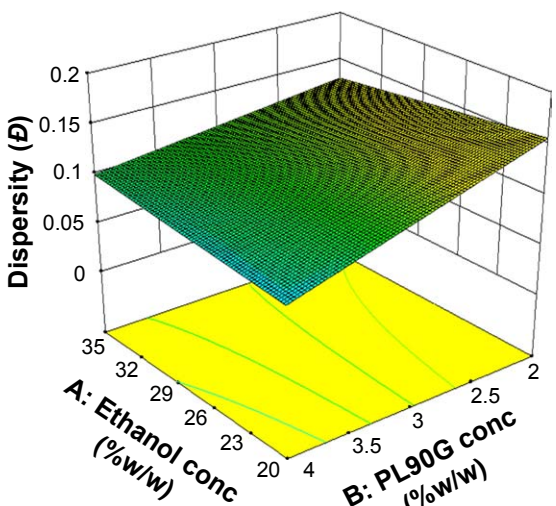


Figure 1 Response surface plots showing influences of the independent factors on the response parameters in TET design.
Abbreviations: PL90G, Phospholipon 90G[®]; T20, Tween 20[®]; TET, transethosomes with Tween 20[®].

Vesicular size



Dispersity



Zeta potential

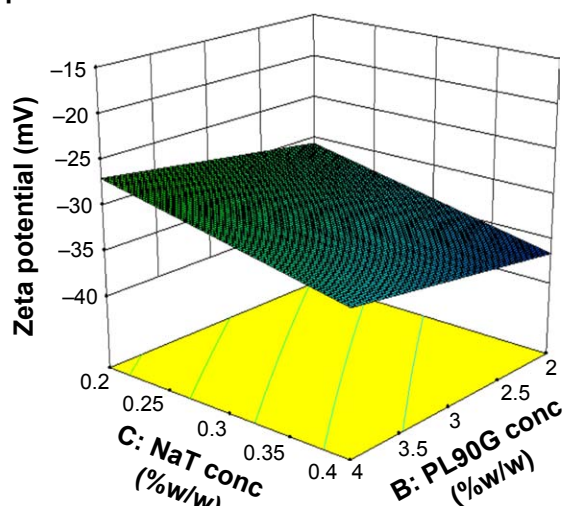
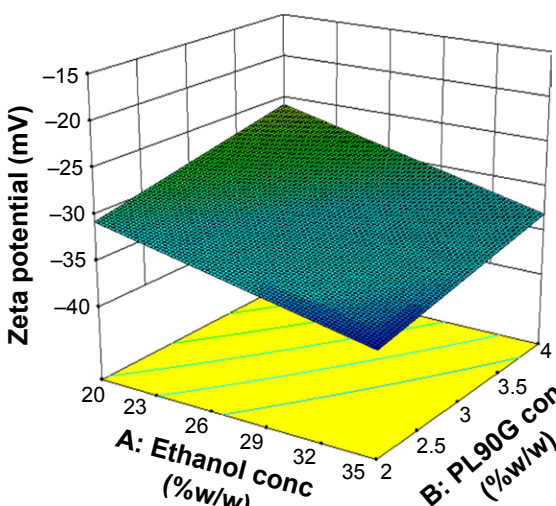


Figure 2 Response surface plots showing influences of the independent factors on the response parameters in TENa design. Abbreviations: PL90G, Phospholipon 90G[®]; NaT, sodium taurocholate; TENa, transethosomes with sodium taurocholate.

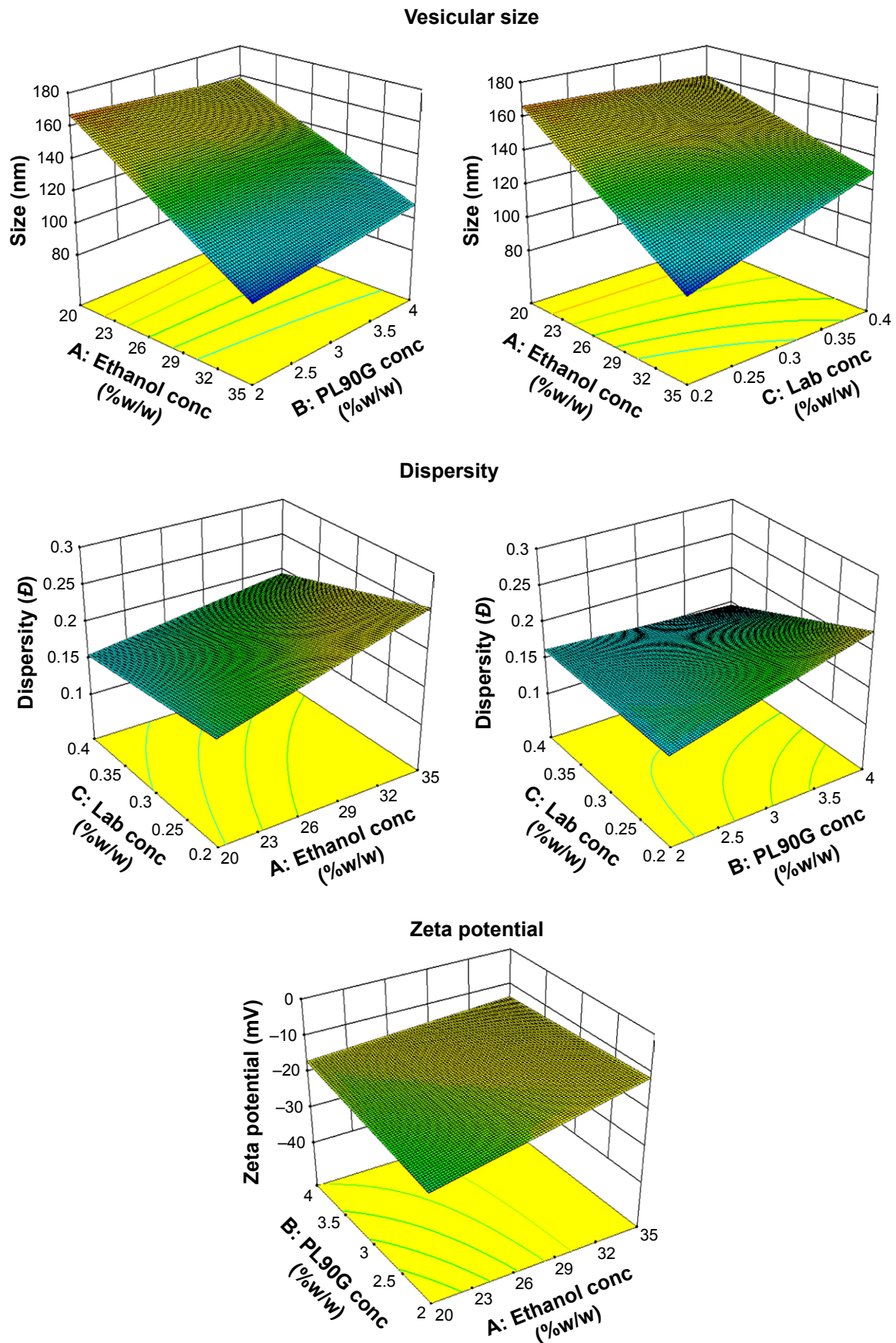


Figure 3 Response surface plots showing influences of the independent factors on the response parameters in TEL design.
Abbreviations: PL90G, Phospholipon 90G®; Lab, Labrafil®; TEL, transethosomes with Labrafil®.

Table 8 The effect (increase/decrease) of individual factors on the vesicular size, \bar{D} , and ZP in each design

Design	Size		\bar{D}		ZP	
	Increase	Decrease	Increase	Decrease	Increase	Decrease
TET	–	A*, B, C*, D*	A*, B	C*, D	A*	B*, C*, D*
TENa	–	A*, B*, C*, D*	C	A, B, D	A*, C*	B*, D*
TEL	C*	A*, B*, D*	A*, B*	C, D*	–	A*, B, C*, D*

Notes: A, B, C, and D refer to the independent variables selected for every factorial design experiment as illustrated in Table 1. *Statistically significant ($p < 0.05$).

Abbreviations: \bar{D} , dispersity; ZP, zeta potential; TET, transethosomes with Tween 20®; TENa, transethosomes with sodium taurocholate; TEL, transethosomes with Labrafil®.

the limit of 35%w/w caused an increase in the vesicular size and led to solubilization of the vesicles. This finding is in agreement with the previously published research regarding the ethanol effects on the vesicular size. The size reduction is probably due to the considerable reduction in vesicular membrane thickness as a result of interpenetration of ethanol hydrocarbon chain in the vesicular lipid bilayers. Ethanol may also cause a modification of the net charge of the system and gives some degree of steric stabilization that may finally lead to a decrease in the mean vesicular size.^{39–42}

Phospholipid concentration was reported to have some effects on the vesicular size, whereby increasing phospholipid concentration would increase the vesicular size.⁴³ Similar effect of the phospholipids on the vesicular size of all the prepared TEs before extrusion was also observed. However, after extrusion, the size of all the vesicles was reduced to <200 nm.

The hydrophilic surfactants Tween 20® (hydrophilic-lipophilic balance [HLB] =16.7) and sodium taurocholate (HLB =16) were found to significantly reduce ($p < 0.05$) the vesicular size of transethosomes with Tween 20® (TET) and TENa TEs, respectively as shown in Figures 1 and 2. These results are in line with other findings reported by some researchers.^{17,44} In contrast, Labrafil® with lower HLB value (HLB =9) was found to have size-increasing effects, whereby increasing Labrafil® concentration increases the vesicular size significantly ($p < 0.05$) as illustrated in Figure 3. This effect might be attributed to the increased hydrophobicity of the surfactant and the interaction of the surfactant with the lipid bilayer. The latter two effects will in turn increase the packing density of the lipid bilayer leading to an increase in the surface free energy and consequently increase the fusion between the lipid bilayer and hence increase the vesicular size.^{45,46}

The number of extrusions was found to have a significant impact ($p < 0.05$) on reducing the vesicular size of all the prepared TEs. Increasing the number of extrusions from 10 to 20 times significantly reduced the vesicular size. Similarly, increasing the number of extrusions also reduced the \bar{D} values of all the formulations.

Effects of the formulation variables on the ZP

Zeta potential is an important parameter that can affect vesicular properties, such as vesicle–skin interaction and TE stability. The results of the zeta potential analysis revealed that all the prepared TEs had negatively charged zeta potential, ranging from –10.58 to –37 mV as shown in Tables 3–5. Ogiso et al reported that the negatively charged vesicles had better skin permeation properties than the positively charged ones.⁴⁷ The negative charge of the zeta potential of ethosomal systems is attributed mainly to the high ethanol content in these nanocarriers. Ethanol provides negative charges to the polar head groups of the phospholipids that would create an electrostatic repulsion. This would reduce the aggregation of ethosomal vesicles and hence enhance the stability of these nanocarriers.

The anionic surfactant sodium taurocholate had a significant ($p < 0.05$) positive impact on the zeta potential values of the prepared TENa formulations by providing negative surface charges to the vesicles. Increasing sodium taurocholate content was associated with a significant increase in the zeta potential values ($p < 0.05$) as illustrated in the ZP equation of the TENa formulations in Table 7 and in the 3D surface plot (Figure 2).

Colchicine incorporation and optimization of EE%

Among all the designs, 23 formulations met the selection criteria of having vesicular size ≤ 150 nm, $\bar{D} \leq 0.3$, and ZP ≥ -20 mV. The selected TEs were loaded with 0.2%w/w of colchicine and then characterized for the vesicular size, \bar{D} , ZP, and EE% (Table 9).

Five formulations namely TET 2-0.2, TET 6-0.2, TET 14-0.2, TENa 4-0.2, and TENa 12-0.2 had EE% $\geq 75\%$, and they were chosen for further optimization. These TEs were loaded with colchicine at concentrations of 0.3 and 0.5%w/w and evaluated for the vesicular size, \bar{D} , ZP, and EE% (Tables 10 and 11).

The vesicular size was still <100 nm when colchicine concentration was increased to 0.3%w/w. However, by

Table 9 Characteristics of transethosomes loaded with 0.2%w/w of colchicine

No	Code	Size (nm)	\mathcal{D}	ZP (mV)	EE%
1	TET 1-0.2	150.8±4.5	0.108±0.003	-22.16±1.35	65.07±0.52
2	TET 2-0.2	92.7±2.9	0.113±0.008	-19.33±2.22	78.54±3.41
3	TET 4-0.2	95.6±1.4	0.106±0.015	-10.11±3.32	65.58±3.35
4	TET 6-0.2	87.5±1.6	0.085±0.009	-18.93±1.37	75.72±2.25
5	TET 8-0.2	93.2±0.4	0.108±0.009	-14.33±2.33	72.06±0.67
6	TET 9-0.2	148.9±3.3	0.130±0.003	-14.63±0.61	72.40±3.75
7	TET 10-0.2	82.7±1.5	0.118±0.003	-18.6±0.49	70.78±5.15
8	TET 14-0.2	79.8±1.9	0.083±0.004	-14.26±2.10	83.57±0.84
9	TENa 2-0.2	95.4±0.4	0.163±0.032	-30.43±0.41	66.26±1.85
10	TENa 3-0.2	145.7±4.9	0.174±0.029	-25.23±1.38	64.30±1.18
11	TENa 4-0.2	96.2±0.8	0.145±0.010	-27.46±3.51	75.21±0.41
12	TENa 6-0.2	99.2±0.8	0.154±0.023	-31.3±3.31	66.52±1.57
13	TENa 7-0.2	141.4±1	0.223±0.012	-26.86±1.30	68.22±0.52
14	TENa 8-0.2	94.2±1.6	0.139±0.014	-31.66±2.49	68.31±0.75
15	TENa 10-0.2	89.9±2.5	0.196±0.017	-27.13±0.20	74.53±3.47
16	TENa 11-0.2	142.6±5.3	0.165±0.027	-22.6±0.96	69.24±1.88
17	TENa 12-0.2	89.9±2.1	0.134±0.012	-20.43±0.47	77.34±0.24
18	TENa 14-0.2	94.8±2	0.149±0.006	-30.16±2.50	71.55±0.63
19	TENa 15-0.2	133.1±2.6	0.153±0.002	-23.56±2.18	66.94±1.73
20	TENa 16-0.2	84.2±0.6	0.133±0.010	-27.46±1.51	67.20±0.52
21	TEL 2-0.2	108.5±1.4	0.240±0.003	-11.73±1.19	72.74±6.08
22	TEL 4-0.2	118.9±2.8	0.183±0.006	-25.13±2.74	60.04±0.52
23	TEL 8-0.2	172.1±20	0.692±0.042	-26.63±1.91	69.07±0.83

Notes: Each value is expressed as mean ± standard deviation (n=3). All formulations shown in bold had EE% values >75% and were selected for further studies.

Abbreviations: \mathcal{D} , dispersity; ZP, zeta potential; EE%, entrapment efficiency; TET, transethosomes with Tween 20®; TENa, transethosomes with sodium taurocholate; TEL, transethosomes with Labrafil®.

further increasing the drug concentration to 0.5%w/w, the vesicular size of all the formulations increased to >100 nm. The \mathcal{D} values of all the selected formulations were <0.3 at all the studied concentrations. The ZP values of the TEs decreased significantly ($p<0.05$) by increasing the drug concentration. The lowest ZP values were observed at a colchicine concentration of 0.5%w/w. Colchicine is highly hydrophilic and a neutral drug, thus it may present in the aqueous phase of the inter-bilayer regions of the vesicles. This will reduce the negative charges generated by ethanol and hence will decrease the ZP values. It was found that increasing colchicine concentration from 0.2 to 0.3%w/w had no statistically significant effects on the EE% of all the formulations, except TET 14-0.3, where the EE% decreased

significantly. However, increasing colchicine concentration further to 0.5%w/w caused a significant decrease ($p<0.05$) in the EE% (<75 %) of all the studied formulations.

Colchicine concentration of 0.2%w/w is too low and is practically not suitable for the next step of the formulation process, which involves the development of transethosomal gels. The drug concentration of 0.5%w/w was also excluded from the selection due to the associated significant ($p<0.05$) increase in the vesicular size and the significant decrease in the EE% ($p<0.05$). Therefore, colchicine concentration of 0.3%w/w was selected as the optimized drug concentration to be loaded into TEs as it produced high EE% and small vesicular size. Accordingly, the TET 2-0.3, TET 14-0.3, TENa 4-0.3, and TENa

Table 10 Characteristics of transethosomes loaded with 0.3%w/w of colchicine

No	Code	Size (nm)	\mathcal{D}	ZP (mV)	EE%
1	TET 2-0.3	91.0±3.6	0.111±0.015	-19.86±0.77	78.84±0.65
2	TET 6-0.3	89.1±4.3	0.131±0.022	-23.00±1.66	73.44±0.57
3	TET 14-0.3	87.6±3.0	0.197±0.040	-15.16±0.57	76.74±0.77
4	TENa 4-0.3	90.4±2.2	0.090±0.004	-24.76±2.29	76.45±0.85
5	TENa 12-0.3	87.6±1.4	0.109±0.009	-20.86±1.09	79.75±1.04

Notes: Each value is expressed as mean ± standard deviation (n=3). All formulations shown in bold had EE% values >75% and were selected for further studies.

Abbreviations: \mathcal{D} , dispersity; ZP, zeta potential; EE%, entrapment efficiency; TET, transethosomes with Tween 20®; TENa, transethosomes with sodium taurocholate.

Table 11 Characteristics of transethosomes loaded with 0.5%w/w of colchicine

No	Code	Size (nm)	\mathcal{D}	ZP (mV)	EE%
1	TET 2-0.5	104.0±0.2	0.138±0.002	-17.00±1.34	69.37±1.46
2	TET 6-0.5	114.1±3.3	0.148±0.02	-18.00±2.08	65.21±0.68
3	TET 14-0.5	110.2±2.8	0.125±0.011	-17.73±1.73	65.34±0.56
4	TENa 4-0.5	142.1±0.6	0.132±0.003	-13.83±1.38	61.83±0.77
5	TENa 12-0.5	138.2±1.3	0.106±0.005	-10.35±1.90	63.98±1.12

Note: Each value is expressed as mean ± standard deviation (n=3).

Abbreviations: \mathcal{D} , dispersity; ZP, zeta potential; EE%, entrapment efficiency; TET, transethosomes with Tween 20[®]; TENa, transethosomes with sodium taurocholate.

12-0.3 formulations (with vesicular size <100 nm and EE% >75%) were chosen for further studies for developing the transethosomal gels.

Preparation of the transethosomal gels

Carbopol[®] polymers (crosslinked polyacrylic acid polymers) are the commonly used gel-forming agents for the incorporation of ethosomal systems. In this study, Carbopol 940[®] was selected because it is compatible with ethosomal nanocarriers and has good bioadhesive properties.⁴⁸ In the preliminary studies of the preparation of colchicine-loaded transethosomal gels, various concentrations (0.5, 1, 1.5, and 2%w/w) of Carbopol 940[®] were tried, but only the concentration of 2%w/w gave suitable consistency (by visual observation) when mixed with the colchicine-loaded transethosomal suspensions.

Methyl paraben and propyl paraben were added to the gel base as preservatives. The four optimized transethosomal formulations (TET 2-0.3, TET 14-0.3, TENa 4-0.3, and TENa 12-0.3) were incorporated into the gel base. The physical appearance of all the prepared colchicine-loaded transethosomal gels was found to be translucent while the prepared NE gel was found to be transparent. The final concentration of colchicine in all the gel formulations was 0.24%w/w; colchicine concentration ≤0.5% is considered safe for topical application.^{12,49} In addition, 0.5 g of the colchicine-loaded transethosomal gel (0.24%w/w) is equivalent to the daily oral dose (1.2 mg) of colchicine.

Characterization of the colchicine-loaded transethosomal gels

The characterization results of the colchicine-loaded transethosomal gels are summarized in Table 12. It was revealed that there was no significant difference between the various gels regarding the vesicular size, \mathcal{D} , ZP, and pH values. However, the ZP magnitude of the transethosomal vesicles was notably increased after their incorporation into the Carbopol 940[®] gel base. This effect might be attributed to the anionic nature of the polyacrylic acid. The pH values of the prepared gels were in the range of 6.72–6.81, which are within the normal pH range of skin, thereby will not cause skin irritation upon application.^{30,50}

The rheological behavior of the prepared gels was studied because it plays an important role in the flow characteristics of the materials during the mixing process, packaging into containers, storage, and application to the skin. The viscosity and yield values of TET transethosomal gels (TET 2-0.3 and TET 14-0.3) were significantly higher than those of TENa transethosomal gels (TENa 4-0.3 and TENa 12-0.3) ($p < 0.05$). This might be attributed to the higher PL90G concentration (4%w/w) in TENa transethosomal gels, in comparison with its concentration (2%w/w) in the TET transethosomal gels. The yield values of all the colchicine-loaded transethosomal gels were in the range of 119.85–131.91 Pa, which are within the desirable yield range (<500 Pa) for the topical drug delivery preparation.³⁰ The rheograms of the various colchicine-loaded transethosomal gels revealed that all the prepared gels exhibited non-Newtonian plastic flow and fitted the Bingham model of rheological behavior. In addition, all the prepared gels exhibited no thixotropy because no hysteresis loops were seen in the rheograms,⁵¹ reflecting no time-dependent decrease in viscosity under stress and stable structure of the polymer used in the preparation, as illustrated in Figure 4.

Among the studied colchicine-loaded transethosomal gel formulations (TET 2-0.3, TET 14-0.3, TENa 4-0.3, and TENa 12-0.3), two formulations (TET 2-0.3 and TENa 4-0.3), which had the highest yield values, were selected for the

Table 12 Characterization of various colchicine-loaded transethosomal gels

Parameter	TET 2-0.3	TET 14-0.3	TENa 4-0.3	TENa 12-0.3
Size (nm)	111.4±1.6	112.7±1.1	110.5±1.1	108.4±1.5
\mathcal{D}	0.157±0.01	0.159±0.012	0.162±0.014	0.153±0.01
ZP (mV)	-40.56±5.32	-41.25±3.24	-43.54±2.56	-43±1.64
pH	6.74±0.05	6.75±0.05	6.81±0.04	6.72±0.03
Viscosity (Pa·s)	66.25±2.75	60.22±0.51	55.68±0.76	53.56±1.43
Yield (Pa)	131.91±1.94	128.55±0.98	122.71±0.99	119.85±1.98

Notes: Each value is expressed as mean ± standard deviation (n=3). All formulations shown in bold were selected for further studies.

Abbreviations: \mathcal{D} , dispersity; ZP, zeta potential; TET, transethosomes with Tween 20[®]; TENa, transethosomes with sodium taurocholate.

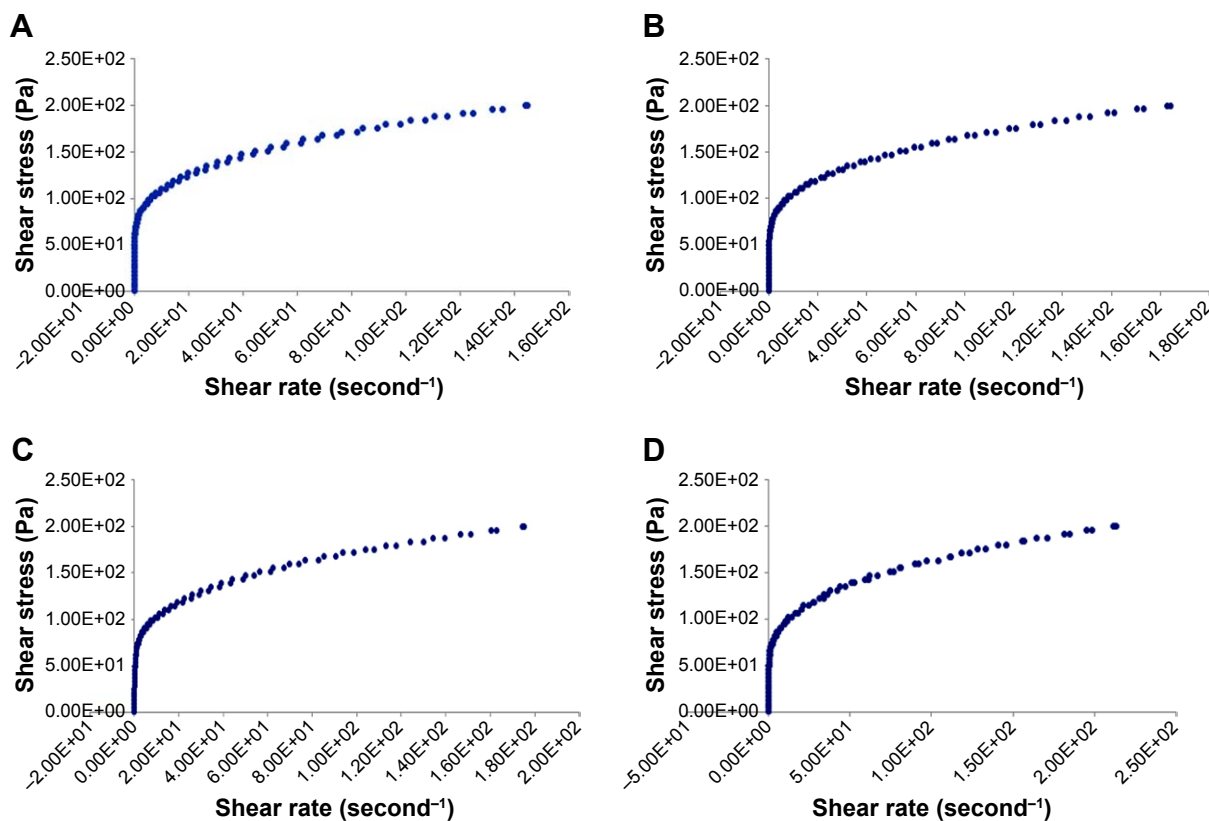


Figure 4 Representative rheograms of the prepared transethosomal gels.
Notes: (A) TET 2-0.3 gel; (B) TET 14-0.3 gel; (C) TENa 4-0.3 gel; (D) TENa 12-0.3 gel.

ex vivo skin permeation study. This is because formulations with higher static yield values are expected to be more stable by maintaining the vesicles in suspension and minimizing sedimentation.

Ex vivo skin permeation studies

The ex vivo skin permeation experiments were conducted using full-thickness-excised rats' back skin to determine

and compare the skin permeation parameters between colchicine-loaded transethosomal gels (TET 2-0.3 and TENa 4-0.3) and the NE colchicine gel formula. All the gels contained the same concentration of the drug (0.24%w/w). The cumulative amounts of colchicine permeated per unit area from the different gel formulations are shown in Figure 5. It was noted that the amounts of colchicine permeated (after 24 hours) through rats' skin from the transethosomal

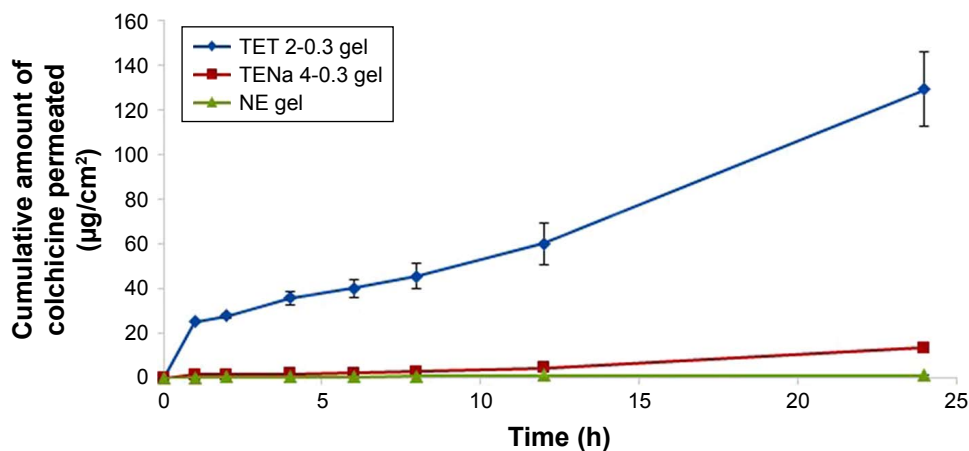


Figure 5 Ex vivo skin permeation of colchicine from various gels.
Note: The data are represented as mean \pm standard deviation ($n=3$).
Abbreviations: NE, non-ethosomal; TET, transethosomes with Tween 20[®]; TENa, transethosomes with sodium taurocholate.

Table 13 The flux at steady state (J_{ss}) and the permeability coefficient (Kp) of colchicine from various gels through rat skin

Code	J_{ss} ($\mu\text{g}/\text{cm}^2/\text{h}$)	Kp (cm/h)
TET 2-0.3 gel	7.233 \pm 1.1	0.0031 \pm 0.0005
TENa 4-0.3 gel	1.084 \pm 0.04	0.0005 \pm 1E-05
NE gel	0.063 \pm 0.01	0.00003 \pm 2E-06

Note: Each value is expressed as mean \pm standard deviation (n=3).

Abbreviations: NE, non-ethosomal; TET, transethosomes with Tween 20[®]; TENa, transethosomes with sodium taurocholate.

gels TET 2-0.3 (129.14 \pm 16.53 $\mu\text{g}/\text{cm}^2$) and TENa 4-0.3 (13.65 \pm 0.45 $\mu\text{g}/\text{cm}^2$) were significantly ($p < 0.05$) higher than the NE gel (1.41 \pm 0.07 $\mu\text{g}/\text{cm}^2$).

The flux (J_{ss}) and permeation coefficient (Kp) values of the tested formulations are summarized in Table 13. There were significant differences ($p < 0.05$) in the flux and Kp values among the studied gels. The highest flux was obtained from TET 2-0.3 gel (7.233 \pm 1.1 $\mu\text{g}/\text{cm}^2/\text{h}$) followed by TENa 4-0.3 (1.084 \pm 0.04), while the flux of the NE gel was 0.063 \pm 0.01 $\mu\text{g}/\text{cm}^2/\text{h}$.

The significantly enhanced skin permeation of colchicine from the transethosomal gels (TET 2-0.3 and TENa 4-0.3) over the NE gel is attributed to the presence of ethanol, phospholipids, and surfactants. These substances are acting synergistically to enhance the skin permeation of colchicine by increasing the malleability of vesicles and the perturbation of the skin lipids, at the same time, leading to a higher number of TEs that reach the deeper layers of the skin and release the drug.^{19,21,44,52}

The type of edge activator used in the preparation of the TEs also played a significant role in enhancing the skin permeation of colchicine from the prepared gels. The TET 2-0.3

gel that contains Tween 20[®] (nonionic surfactant, HLB=16.7) had significantly better skin permeation properties than TENa 4-0.3 gel that contains sodium taurocholate (anionic surfactant, HLB=16). This could be due to the higher vesicular deformability provided by Tween 20[®] than for sodium taurocholate, leading to higher skin permeation. Tween 20[®] was reported to provide significantly higher vesicular deformability in comparison to sodium taurocholate and other bile salts in retinol-loaded deformable liposomes.⁵³ Based on the obtained results, TET 2-0.3 gel was selected as the optimized colchicine-loaded transethosomal gel formulation and was subjected to further studies of TEM and stability.

TEM

The morphology of the optimized TET 2-0.3 formulation was visualized by the TEM in the initial suspension form and after incorporation into the gel base, as shown in Figure 6. The TEs of TET 2-0.3 formulation in both the forms had irregular spherical shape. This might be attributed to the presence of surfactant (Tween 20[®]) in the composition of the vesicles which perturbed the lipid bilayer and increased their fluidity.¹⁷

Stability studies

The results of the stability studies of TET 2-0.3 gel in both the conditions, 4°C \pm 2°C and 25°C \pm 2°C/60% \pm 5% RH, are summarized in Tables 14 and 15. The physical stability of TET 2-0.3 formulation was observed visually. It was found that the appearance of the TET 2-0.3 gel formulation was unchanged during the 3 months of storage at the refrigerated (4°C \pm 2°C) or room temperature (25°C \pm 2°C/60% \pm 5% RH) conditions.

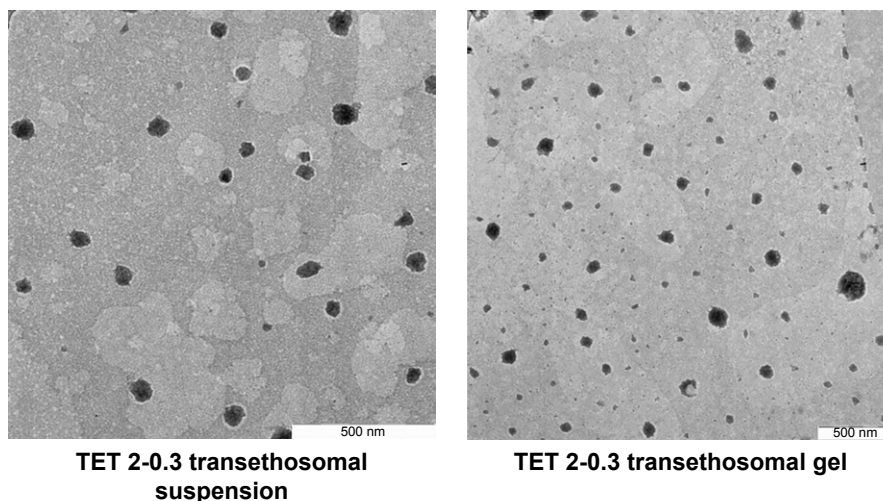


Figure 6 TEM images of TET 2-0.3 transethosomes in its suspension and transethosomal gel forms.

Abbreviations: TEM, transmission electron microscopy; TET, transethosomes with Tween 20[®].

Table 14 Stability study results of the TET 2-0.3 gel stored at 4°C±2°C

Parameters	0 months	4°C±2°C		
		1st month	2nd month	3rd month
Size (nm)	111.4±1.6	119.4±1.9	130.5±1.8	141.7±1.8
Đ	0.157±0.01	0.145±0.013	0.152±0.015	0.156±0.012
ZP (mV)	-40.56±5.32	-39.2±2.23	-38.3±4.64	-47.3±1.2
pH	6.74±0.05	6.77±0.14	6.8±0.04	6.88±0.09
Drug content	99.94±0.32	99.98±0.36	99.06±1.16	99.16±0.47
Viscosity (Pa·s)	66.25±2.75	66.84±2.36	66.69±2.48	66.81±1.9
Yield (Pa)	131.91±1.94	132.17±1.38	131.6±1.26	132.03±1.25

Note: Each value is expressed as mean ± standard deviation (n=3).

Abbreviations: Đ, dispersity; ZP, zeta potential; TET, transethosomes with Tween 20®.

The vesicular size of TET 2-0.3 gel was found to increase slightly, by ~30 nm only, in the overall 3 month storage period at the refrigerated storage condition (4°C±2°C). However, this increment was found to be statistically significant ($p < 0.05$). On the other hand, the vesicular size of TET 2-0.3 gel samples stored at 25°C±2°C/60%±5% RH was increased by ~30 nm in the first month and ~50 nm in the overall 3 months of storage. There were no significant changes ($p > 0.05$) in Đ and ZP values during the 3 month study period under both the tested storage conditions.

The pH values of the TET 2-0.3 gel samples remained stable at the refrigerated storage condition for the 3 month period. Similarly, no changes in the pH were observed in the first 2 months at the room temperature condition, but a slight increase in pH occurred in the third month. However, all the pH values under both the storage conditions and over the 3 month period were within the normal pH range of the skin.

No significant ($p > 0.05$) variation in drug content was observed over the 3 months of the stability study in the refrigerated storage condition. In contrast, the drug content in the samples stored at room temperature was stable in the

Table 15 Stability study results of the TET 2-0.3 gel stored at 25°C±2°C/60%±5% RH

Parameters	0 months	25°C±2°C/60%±5% RH		
		1st month	2nd month	3rd month
Size (nm)	111.4±1.6	140.7±1.4	150.1±1.3	162.1±2.1
Đ	0.157±0.01	0.173±0.013	0.155±0.021	0.176±0.021
ZP (mV)	-40.56±5.32	-36±2.62	-45±2.57	-44.4±1.35
pH	6.74±0.05	6.76±0.11	6.85±0.05	7.07±0.14
Drug content	99.94±0.32	99.34±0.4	97.62±0.74	95.96±0.61
Viscosity (Pa·s)	66.25±2.75	66.86±2.12	66.51±1.92	66.01±2.6
Yield (Pa)	131.91±1.94	131.94±1.11	131.54±1.31	132.01±2.22

Note: Each value is expressed as mean ± standard deviation (n=3).

Abbreviations: Đ, dispersity; ZP, zeta potential; RH, relative humidity; TET, transethosomes with Tween 20®.

first month only, thereafter it started to decrease significantly ($p < 0.05$). The decrease in drug content might be due to the chemical degradation of the phospholipids in the transethosomal vesicles, which disturbed the membrane packing, and eventually led to drug leakage.⁵⁴ No significant changes were observed in the viscosity, yield values, and the rheological behavior of the TET 2-0.3 gel samples under both the tested storage conditions and over the 3 month period of the stability study.

Conclusion

Colchicine-loaded transethosomal gels were successfully developed and optimized using three 2⁴ full factorial experimental designs. The optimized formulation had suitable characteristics for the transdermal delivery of drugs, such as small vesicular size, negatively charged ZP, and high EE%. The ex vivo skin permeation studies revealed that the transethosomal gels had superior skin permeation properties in comparison to the NE gel formulation. The stability studies showed that the optimized colchicine-loaded transethosomal gel had satisfactory stability at the refrigerated condition (4°C±2°C). Therefore, it is concluded that transethosomal gels are potential carriers for the transdermal delivery of colchicine. These carriers provide an alternative route for the administration of this drug to overcome the poor bioavailability and severe side effects associated with the oral route.

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Disclosure

The authors report no conflicts of interest in this work.

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