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Research Article

Optimized Transethosomal Carrier of Psoralen for Enhanced Topical Psoriasis Treatment: In-vitro, In-vivo, and Stability Evaluation

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ABSTRACT

Background: The optimized transethosomal (TE) formulation of the psoralen was developed using statistical design of experiment tool for its effectiveness against psoriasis.

Material and Methods: Psoralen-encapsulated transethosomes were prepared by "cold method. With due diligence, the selected Critical process parameters were concentration of lipid, surfactant & ethanol; temperature and stirring. The design of experiment was planned using half fraction design with 2 blocks. The targeted critical attributes of quality were vesicle size & deformability, zeta potential, entrapment efficiency. The optimized formulation was evaluated for the *in vitro* release profile utilizing the Franz's diffusion cell and further evaluated for antipsoratic activity *in vivo* Imiquimod induced animal model of psoriasis.

Results: In case of vesicle size the impact of D i.e. temperature and square of C (Ethanol Conc.), D and E (Stirring rate) were significant (<0.05) while overall lack of fit was significant. Whereas, in the case of zeta potential A (PC Conc.) was only significant parameter with insignificant lack of fit. The optimized formulation was characterized with FTIR, Transmission electron microscopy and zeta potential. *In vitro* drug penetration study expressed that TE-psoralen 68.32 ± 4.87 % release without saturation even after 24 h which was more than psoralen free with saturation. *In vivo* and ILs expression studies were in parallel with enhanced release results and expressing the efficacy of TE-Psoralen 50 µM equivalent to free psoralen 1 mM concentration.

Conclusion: TE-Psoralen produced were having good stability, higher deformability thus efficacy for skin related indication.

Keywords Psoralen, Transethosomes, RSM, Design of Experiment, Statistical Optimization Tool, *In Vitro* and *In Vivo* Evaluation, Psoriasis

Introduction

Psoriasis manifests as a chronic condition marked by persistent discomfort, resulting in somatic distress typified by intense pain, accompanied by a pronounced exacerbation of psychological anguish and societal ostracization for the afflicted individual, precipitating a profound deterioration in their quality of life, concomitant with depressive symptoms and significant socioeconomic encumbrance attributable to the financial exigencies of treatment.

Within the annals of medical discourse, this ailment stands as an affliction devoid of definitive remedy, with therapeutic interventions limited to palliative measures. Its peak incidence, as documented, reached 11.4% in Norway, prompting the World Health Organization (WHO) to designate it a pervasive global concern in 2014, compelling the formulation of a comprehensive report aimed at fostering awareness and providing guidance to policymakers .

The pathophysiological elucidation of psoriasis unveils the intricate engagement of diverse cytokines, including tumor necrosis factors (TNFs) and interleukins, mediated through T-helper cells and/or Janus kinase-signal transducer and activator of transcription (Jak-STAT) pathways, exhibiting variable degrees of involvement (Srivastav et al. 2009, Abdallah, Elghamry et al. 2023).

Psoralen, a small molecule that belongs to the coumarin family of compounds (Zhang, Shen et al. 2014). The fundamental chemical scaffold entails a furan ring fused to a coumarin nucleus, with potential additional substituents bestowing a spectrum of pharmacological functionalities. These bioactive agents occur naturally across an array of botanical taxa, encompassing citrus fruits, parsley, and figs. Moreover, they are synthesized synthetically to serve therapeutic and investigational endeavors within the medical domain (Pathak and Fitzpatrick 1992).

Chemical name of Psoralen is "6,7-Furanocoumarin; 7H-Furo[3,2-g]benzopyran-7-one; Ficusin; Furo[3,2-g]coumarin" **(Figure 1).** Psoralen exhibits several pharmacological activities, mainly due to its interaction with DNA and its photosensitizing properties like phototoxicity (Stern and Study 2012), antimicrobial activity (Siva, Sivakumar et al. 2015), anti-inflammatory (Li, Xu et al. 2021) and anticancer acitivity (Dwivedi et al. 2024).

Psoralen may cause systemic side effects when administered orally or systemically, including gastrointestinal disturbances, liver toxicity, and neurological symptoms. These side effects limit its use in systemic treatments and require cautious dosing and monitoring (Stern 2007). Psoralen has low topical bioavailability. Psoralen's effective skin penetration is crucial for reaching its target cells. Psoralen has poor topical bioavailability (Zhang, Shen et al. 2014). Nonetheless, its hydrophobic nature and large molecular size often hinder skin permeation (Prausnitz and Langer 2008).

To address this, various formulation strategies have been explored, including the utilization of penetration enhancers, nanoemulsions, or encapsulation within liposomes, which can enhance its skin penetration capabilities.

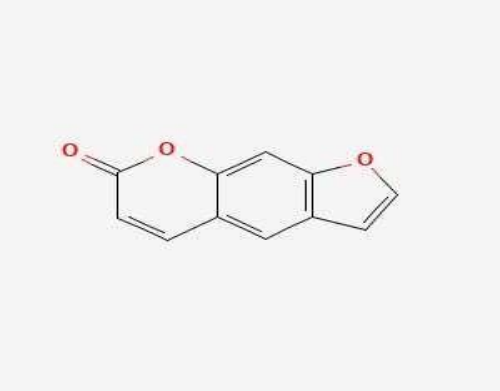


Figure 1. Chemical Structure of Psoralen. Source pubchem

In case of psoriasis the majorly focused site of action appears to be topical. Conventional topical system for example lotion, cream, gels, ointments represented many limitations in terms of deeper penetration of the skin. The problem of incomplete penetration can be overcome by the use of vesicular drug delivery systems such as liposomes, transfersomes, niosomes, and ethosomes (Garg, Singh et al. 2017). Ethosomes are phosphatidylcholine (PC)-based colloidal nanosystems arranged as multilamellar vesicles that contain significant concentrations of ethanol (20–45%) (Natsheh, Vettorato et al. 2019). Since the ethanol stabilizes the vesicles while enhancing their softness and capacity to carry lipophilic medicines, these nanosystems are more thermodynamically stable than liposomes (Jain, Tiwary et al. 2007, Shen, Zhang et al. 2014, Touitou and Natsheh 2021). Despite the ethosomes potential for transdermal administration, the scientists explained that it was necessary to combine their topical application with iontophoresis in order to do so (Mombeiny, Tavakol et al. 2021).

Transethosomes (TEs) are vesicular systems having a high ethanol concentration, phospholipid content, and one or more edge activators, such as sodium deoxycholate. These can be visualized as a combination of transferosome and ethosomes. Trans-ethosomes showed both the quality of deformability and skin permeation. This vesicular system was introduced in the year 2012 by Song et al (Song, Wen et al. 2019). TEs alter the composition of ethosomes by adding edge activators such surfactants, which increases the

effectiveness of drug encapsulation and the penetrating ability of vesicles (Chen, Li et al. 2017, Verma and Utreja 2018, Sindhu, et al. 2021). For instance, phosphatidylcholine in an ethanol solution can be combined with polysorbate 80 to create vesicles with the size, morphology, and deformability necessary for transdermal penetration once applied to the skin (Sguizzato, Ferrara et al. 2021). TEs are suitable for topical as well as systemic route, for entrapment of drugs of low molecular weight to high molecular weight. As the bioactive agent is protected due to encapsulation, so it releases its content in a very slow and gradual manner which in turn can be controlled by formulation ingredients and parameters. Due to simple and scalable preparation, biodegradable and biocompatible nature, TE is a candidate of preference in drug delivery system.

The global regulatory authorities are emphasizing to develop the process not with respect to one factor at a time (OFAT) approach but creating design space within which the process will be called same if any changes are required and lies within design space. Such a design space is a product of statistical design of experiment (DoE) i.e. response surface methodology (RSM). RSM approach in DoE for the development and optimization of pharmaceutical processes provided the outcomes in minimum experimentation and time. Hence such regulatory acceptable approach not only proven to be far more efficient and cost effective than the conventional OFAT methods but also provide the convenience to the organization for being flexible in the process (Goyal, Mishra et al. 2022, ICH-Q9-(R1) 2023).

Considering above principles as background, the present work included the transethosomal formulation of Psoralen using DoE (RSM) in order to enhance the effectiveness for the indication of psoriasis. In order to boost the psoralen's bioavailability thus efficacy, an effort was made in the current work to create a topical drug delivery method.

Methodology

Materials

Psoralen was procured from Sigma Aldrich Pvt. Ltd.Mumbai. Phosphatidyl Choline (PC), Sodium cholate (NaCo) were purchased from Loba Chem Pvt. Ltd. Mumbai. Dialysis Membrane (12-14 k Dalton,M.W.) was procured from Hi media Pvt.Ltd. Mumbai. Betamethasone cream (Betnovate, 0.1%, GSK), Imiquad cream (Imiquimod 5%, Glenmark), IL-17 and IL-23 ELISA kits (Krishgen Biosystem) were also procured. Rest of chemicals and solvents used in this investigation were of analytical or HPLC grade.

Preparation of psoralen loaded transethosomes

Utilizing a method derived from established literature (Ascenso, Raposo et al. 2015), TEs were prepared through a specialized "cold method." The process involved dissolving 5 mg/mL of psoralen alongside phosphatidyl choline (PC) and sodium cholate (NaCo) in ethanol. Water was then added drop by drop via a peristaltic pump until reaching a total volume of 10 mL. This entire procedure was conducted at a controlled temperature of 30 ±4 °C, with continuous magnetic stirring for 30 minutes. The quantities of each component were carefully selected based on the experimental design, maintaining a consistent psoralen concentration throughout.

Design of Experiment

Five key critical process parameters (CPPs) crucial to the preparation of TEs were the concentrations of PC, NaCo, and ethanol; as well as the temperature and stirring conditions. For our experimental design, we chose the Response Surface Method using a Randomized Central Composite Design. Importantly, all five CPPs were treated as numeric factors rather than categorical ones, as detailed in **Table *1***. To optimize experiments efficiently, a half-fraction design across two blocks, totaling 36 runs was employed. Block 1 comprised 16 non-center points and 8 center points, while Block 2 had 10 non-center points and 2 center points.

Design Expert software facilitated the generation of a comprehensive set of combinations for all factors, as outlined in **Table 2**. This systematic approach ensures thorough exploration and understanding of the interactions between these critical parameters to achieve the desired outcomes in our TEs preparation.

Table 1. Input for experiment design independent variables i.e. CPPs for preparation of TEs loaded with psoralen

CPP	Units	Low (-1 code)	High (+1 code)	-alpha	+alpha
PC Conc. (A)	mg/mL	0.5	5	-1.75	7.25
NaCo Conc. (B)	mg/mL	0.1	1	-0.35	1.45
Ethanol (C)	%, v/v	5	50	-17.5	72.5
Temperature (D)	°C	10	50	-10	70

Stirring (E)	Rpm	50	250	-50	350

CPP: Critical Process Parameters, PC: Phosphatidyl choline, NaCo: Sodium Cholate

Table 2. The experiment combinations of CPPs given by design expert

Block	Run	A:PC Conc.	B:NaCo Conc.	C:Ethanol	D:Temperature	E:Stirring
		mg/mL	mg/mL	%, v/v	°C	Rpm
Block 1	1	5	1	50	50	250
Block 1	2	2.75	0.55	27.5	30	150
Block 1	3	5	1	5	10	250
Block 1	4	0.5	1	50	10	250
Block 1	5	2.75	0.55	27.5	30	150
Block 1	6	0.5	1	5	50	250
Block 1	7	0.5	0.1	50	50	250
Block 1	8	2.75	0.55	27.5	30	150
Block 1	9	2.75	0.55	27.5	30	150
Block 1	10	5	0.1	50	10	250
Block 1	11	2.75	0.55	27.5	30	150
Block 1	12	5	0.1	5	10	50
Block 1	13	0.5	0.1	5	10	250
Block 1	14	2.75	0.55	27.5	30	150
Block 1	15	2.75	0.55	27.5	30	150
Block 1	16	2.75	0.55	27.5	30	150
Block 1	17	5	1	50	10	50
Block 1	18	5	0.1	50	50	50
Block 1	19	0.5	1	5	10	50
Block 1	20	0.5	0.1	50	10	50
Block 1	21	0.5	1	50	50	50
Block 1	22	5	1	5	50	50
Block 1	23	0.5	0.1	5	50	50
Block 1	24	5	0.1	5	50	250
Block 2	25	2.75	0.55	27.5	30	150
Block 2	26	2.75	0.55	27.5	70	150
Block 2	27	2.75	0.55	27.5	30	350
Block 2	28	7.25	0.55	27.5	30	150
Block 2	29	2.75	0.55	72.5	30	150
Block 2	30	2.75	0.55	27.5	30	150
Block 2	31	2.75	0.55	-17.5	30	150
Block 2	32	2.75	0.55	27.5	30	-50*
Block 2	33	2.75	-0.35*	27.5	30	150
Block 2	34	2.75	0.55	27.5	-10**	150
Block 2	35	2.75	1.45	27.5	30	150
Block 2	36	-1.75*	0.55	27.5	30	150

CPP: Critical Process Parameters, PC: Phosphatidyl choline, NaCo: Sodium Cholate

* the negative value has been taken as zero due to impracticality

** the negative value was taken as 10 due to impracticality

Drug entrapment efficiency

In accordance with the Design Expert proposed set of formulation, each formulated sample was meticulously assessed for its entrapment efficiency wrt psoralen. The evaluation method entailed centrifugation at 15,000 x g using a Remi-03 Plus centrifuge at 10°C. The psoralen content in the supernatant was then quantified using the analytical method described in the subsequent subsection.

$$\%EE = \frac{PS_T - PS_S}{PS_T} \times 100 \qquad (1)$$

where, EE is entrapment efficiency, PS_T is amount of Psoralen added in the reaction, PS_S is the amount of Psoralen present in the supernatant.

Vesicle deformability (VD)

To evaluate the deformability of the TEs, the extrusion method was employed. The TEs were extruded through a polycarbonate membrane with a pore diameter of 50 nm, mounted on a 25 mm diameter filter holder. Applying a pressure of 2.5 bar at room temperature for one minute, we extruded the TE suspension. Subsequently, the volume and vesicle size of the extruded TEs suspension were quantified.

The Vesicle Deformation (VD) was calculated using the equation provided below:

$$DV = Jx\frac{VS}{PS} \qquad (2)$$

where DV is the vesicle deformability; J is flux of extrusion i.e. the ratio of volume (mL) and time (min) of extrusion; VS is the vesicle size (nm) of formulation extruded; and PS is the pore size of the polycarbonate membrane filter (Esposito, Calderan et al. 2022).

Zeta potential, Particle Size and Polydispersity Index

To determine the particle charge, a Zetasizer (HORIBA ZS 100) operating at 25 °C was utilized. The measurement was conducted by assessing light scattering at an electrode voltage of 3.3 V, employing a blend of laser Doppler velocimetry and phase analysis. The viscosity of the dispersion medium was established at 0.896 mPas. Dynamic light scattering, based on light scattering principles, was employed to estimate the mean particle size using a HORIBA PS 100 Particle Sizer at 25 °C, with measurements taken in triplicate. Prior to measurement, samples were appropriately diluted with distilled water to ensure accurate readings.

Transmission electron microscopy (TEM) evaluation

The size of the TEs was further characterized using Transmission Electron Microscopy (TEM) with a Hitachi H-7500 operating at 120 kV. Approximately 50 µL of both blank TEs and psoralen-loaded TEs samples were deposited onto copper grids. Subsequently, the grids were stained with a 1% phosphotungstic acid solution. After a 5-minute incubation period, excess staining solution was carefully removed by blotting with filter paper, followed by air drying. Images of the samples were captured at magnifications ranging from 10,000X to 100,000X at 80 kV, providing detailed insights into the morphology and size distribution of the TEs.

FTIR study

Fourier Transform Infrared (FTIR) spectra of the pure drug, individual excipients, physical mixture, and the optimized TE formulation were acquired using a BRUKER Alpha II FTIR Spectrophotometer. Approximately 5 mg of each sample was carefully placed for analysis. Spectra were recorded over a wavenumber range of 400-4000 cm^{-1} to identify and characterize the functional groups present in the samples.

Chromatographic analysis of sample by using HPLC method

The psoralen analysis was carried out with the method mentioned in the literature (HPLC Method for Simultaneous Quantification of Bakuchiol and Minor Furocoumarins in Bakuchiol Extract from Psoralea corylifolia). The method was reverse-phase high-performance liquid chromatography (HPLC) equipment, specifically the LC-20 AD system from Shimadzu. In this analysis, Luna phenyl-hexyl column (150 × 4.6 mm id and 5 µm) was used as stationary phase with compatible guard column. The initial ratio of mobile phase A (water) and B (methanol) 45:55 (A:B, v/v) was kept for the first 8 min at constant flow rate 1 mL/min then moved towards organic methanol 100 % in a ramp of 12 min followed by holding for another 5 min. Before next injection the column was equilibrated initial ratio of mobile phase for 5 min. The wavelength taken was 246 nm with injection volume 20 µL.

The calibration curve generated for psoralen exhibited linearity within the concentration range of 0.1-2 µg/mL, with an r-squared (r^2) value of 0.9982. The psoralen eluted out from column at the chromatographic conditions mentioned above at retention time of 6.3 minutes.

Stability Study

The physical stability of the psoralen transethosomal formulation was rigorously evaluated over a two-month period. The formulations were stored in 10 mL coloured glass vials and refrigerated at a controlled temperature of 4±2°C to mimic storage conditions. At the end of each month, aliquot samples were withdrawn from each batch for spectrophotometric analysis to quantify the remaining psoralen content. Additionally, the Zetasizer NanoZS from HORIBA Instrument, Japan, was employed to monitor any changes in the size and size distribution of the TEs. Furthermore, a macroscopic visual inspection was conducted on

select transethosomal formulations to detect any signs of sedimentation or alterations in color, providing supplementary data on the formulation's physical integrity over the storage period.

In-Vitro drug release study

In the donor compartment of the diffusion cell, various amounts of transethosomal formulations, each weighing 4 mg, were introduced. The receptor medium in the receptor compartment consisted of phosphate buffer solution with a pH of 7.4. A semi-permeable cellulose dialyzing membrane (Dialysis Membrane 12,000-14,000 MW cut-off) was utilized to separate the donor and receptor compartments, having been preconditioned by soaking in PBS pH 7.2 for 24 hours. Throughout the experiment, the medium in the receptor compartment was stirred at 50 rpm on a magnetic stirrer, maintaining a constant temperature of 37°C to simulate physiological conditions. To ensure sink conditions, 3 mL aliquots of samples were periodically withdrawn and replaced with fresh PBS solution. Subsequently, the collected samples were appropriately diluted and subjected to analysis for drug release using an HPLC method. This comprehensive approach allowed us to evaluate the in-vitro release kinetics of the transethosomal formulations accurately.

In vivo anti-psoriasis evaluation of psoralen loaded Transethosomes

The experiment on animal was carried out with standard conditions and environment as suggested by the Committee for the purpose of control and supervision on experimental animals (CCSEA) appropriate approval (reference number IAEC/74/1451). Animals were acclimatized in the experimental area for two days in order to reduce the bias in the results.

Imiquimod based psoriasis like skin inflammation model in mice was referred from literature (More, Sharma et al. 2021). In brief, mice of 20-27 g were shaved on the dorsal part of the body and approximately 62.5 mg/d of 5% Imiquimod cream (eq. to 3.125 mg of Imiquimod) was applied for 6 days.

The animals were grouped into 6 groups (n=6) viz Sham only shaving on dorsal body (No IMQ), negative control group given 40% ethanol only (Cont), transethosome control (TE-Cont), positive control of standard i.e. betnovate cream (STD), Psoralen solution (1 mM) and three treatment groups 50, 100, 500 µM psoralen transethosome suspension. Each group were treated with equal volume or equivalent mass of the solutions/suspensions/cream as per the formulation.

Psoriasis Area Severity Index (PASI) score i.e. 0- no observation, 1- mild, 2- moderate, 3- severe, 4- very severe was established with respect to skin thickness & scaling and erythema with the support of observer kept blinded for the study at different days interval with the variation of ±2 hours.

Biochemical Estimation

Interleukins were extracted from homogenized skin tissues with buffer for extraction from ELISA kit. The skin samples were weighed followed by their homogenization using ELISA extraction buffer containing protease and phosphatase inhibitors, and centrifugation at 4 °C for performing ELISA. The acquired tissue supernatants were utilized to estimate cytokine levels including IL-17 and IL-22, using commercially available ELISA kits.

Results and discussion

Analysis of results obtained in DOE

To enhance product quality, leveraging the Design of Experiments (DOE) is essential. By manipulating independent factors strategically, DOE facilitates the creation of top-tier products, tailored for optimal results. This method revolutionizes manufacturing, ushering in unprecedented excellence. Authors, experts, and literature collectively guided the assessment of parameters affecting TE vesicles, such as size, zeta potential, entrapment efficiency, and deformability. Critical factors identified included PC, NaCo, ethanol concentration, temperature, and stirring rate. These insights guided the DOE application, optimizing outcomes. Experimentation, guided by DOE, targeted vesicle size, zeta potential, entrapment efficiency, and deformability, shaping a future of refined product development.

The statistical evaluation of results yielded polynomial equations (Equations 3-6) via software analysis. These equations not only quantify the parameter effects but also indicate whether they exert agonistic or antagonistic impacts, inferred from the equation signs. Fit summaries revealed all factors followed a quadratic model, with zeta potential and vesicle deformability also displaying linear tendencies. However, ANOVA analysis was exclusively conducted on the quadratic model. This meticulous statistical scrutiny provides comprehensive insights into parameter influences, crucial for understanding and optimizing the experimental outcomes.

$$\text{Vesicle Size (nm)} = 102.1 + 1.8\,A - 7.75\,B - 4.5\,C - 32.9\,D - 12.5\,E - 4.5\,AB + 3.4\,AC + 2.9\,AD + 2.1\,AE + 0.5\,BC - 10.3\,BD - 5.0\,BE + 10.4\,CD - 3.6\,CE - 0.13\,DE + 26.6\,A^2 + 8.7\,B^2 + 55.9\,C^2 + 43.5\,D^2 + 30.2\,E^2 \quad \ldots\ldots\ldots (3)$$

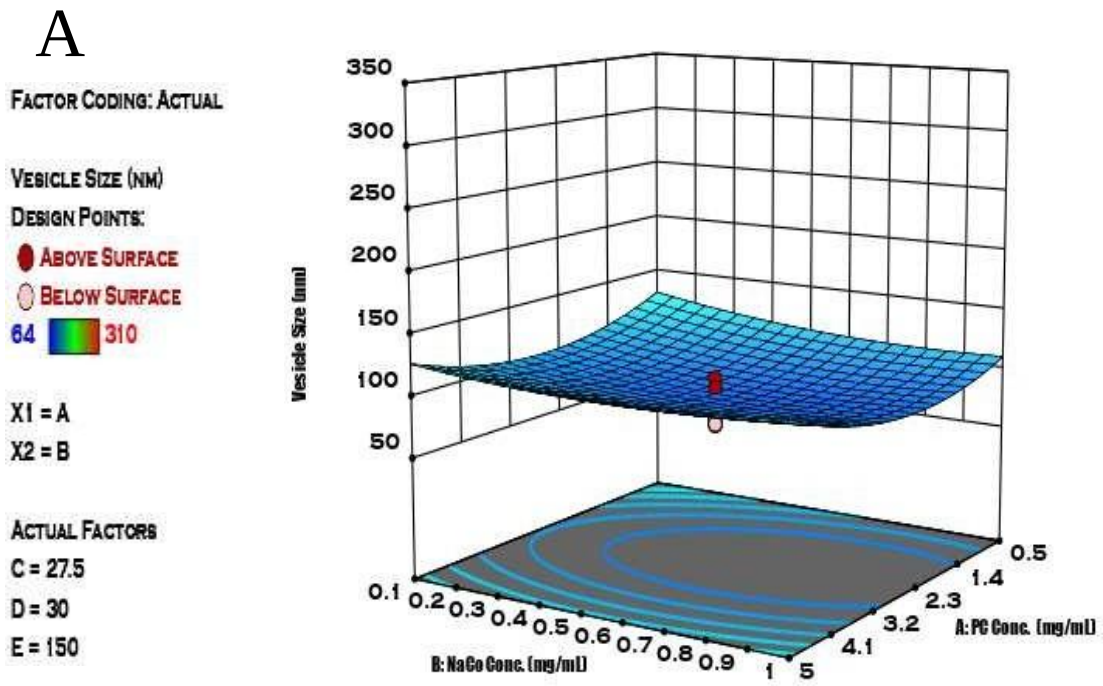
$$\text{Zeta Potential (mV)} = -17.6 + 4.0\,A + 1.7\,B + 1.1\,C - 0.95\,D + 0.52\,E \quad \ldots\ldots\ldots (4)$$

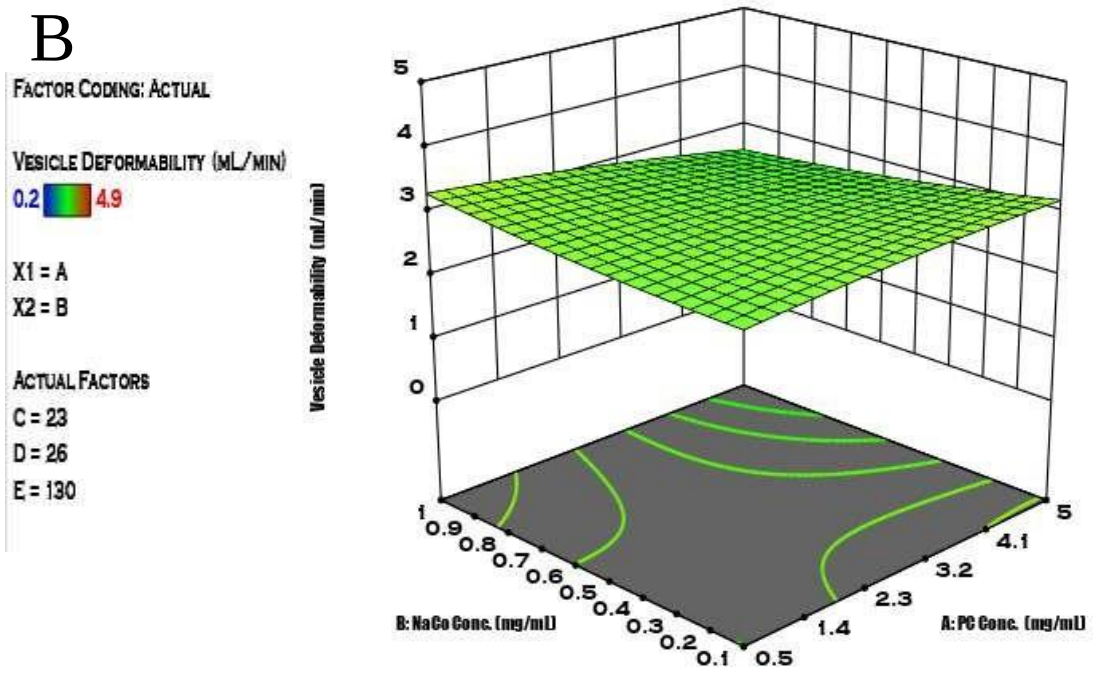
$$\text{Entrapment Efficiency (\%)} = +35.2 - 3.9\,A - 1.2\,B - 4.1\,C + 1.5\,D + 1.3\,E \quad \ldots\ldots\ldots (5)$$

$$\text{Vesicle Deformability} = -3.8 - 0.02\,A - 0.95\,B + 1.6\,C + 0.16\,D - 0.06\,E - 0.38\,AB + 0.12\,AC - 0.08\,AD - 0.09\,AE - 0.19\,BC - 0.24\,BD - 0.23\,BE + 0.31\,CD + 0.09\,CE - 0.06\,DE - 0.03\,A^2 + 0.08\,B^2 - 0.58\,C^2 + 0.2\,D^2 - 0.02\,E^2 \quad \ldots\ldots\ldots(6)$$

where, A is PC Conc. (mg/mL), B is NaCo Conc. (mg/mL), C is Ethanol Conc. (%, v/v), D is Temperature (°C) and E is Stirring speed (rpm).

The impact of CPP/s was presented in statistical form i.e. ANOVA which showed that overall quadratic model for vesicle size, zeta potential and vesicle deformability which was significant. For the zeta potential and entrapment efficiency suggested model was linear, however the model was insignificant for the entrapment efficiency attribute. In case of vesicle size the impact of D i.e. temperature and square of C (Ethanol Conc.), D and E (Stirring rate) were significant (<0.05) while overall lack of fit was significant. Whereas, in the case of zeta potential A (PC Conc.) was only significant parameter with insignificant lack of fit. The outcome from the Design Expert in form of 3D graphs showing design space are presented in the **Figure 2** .





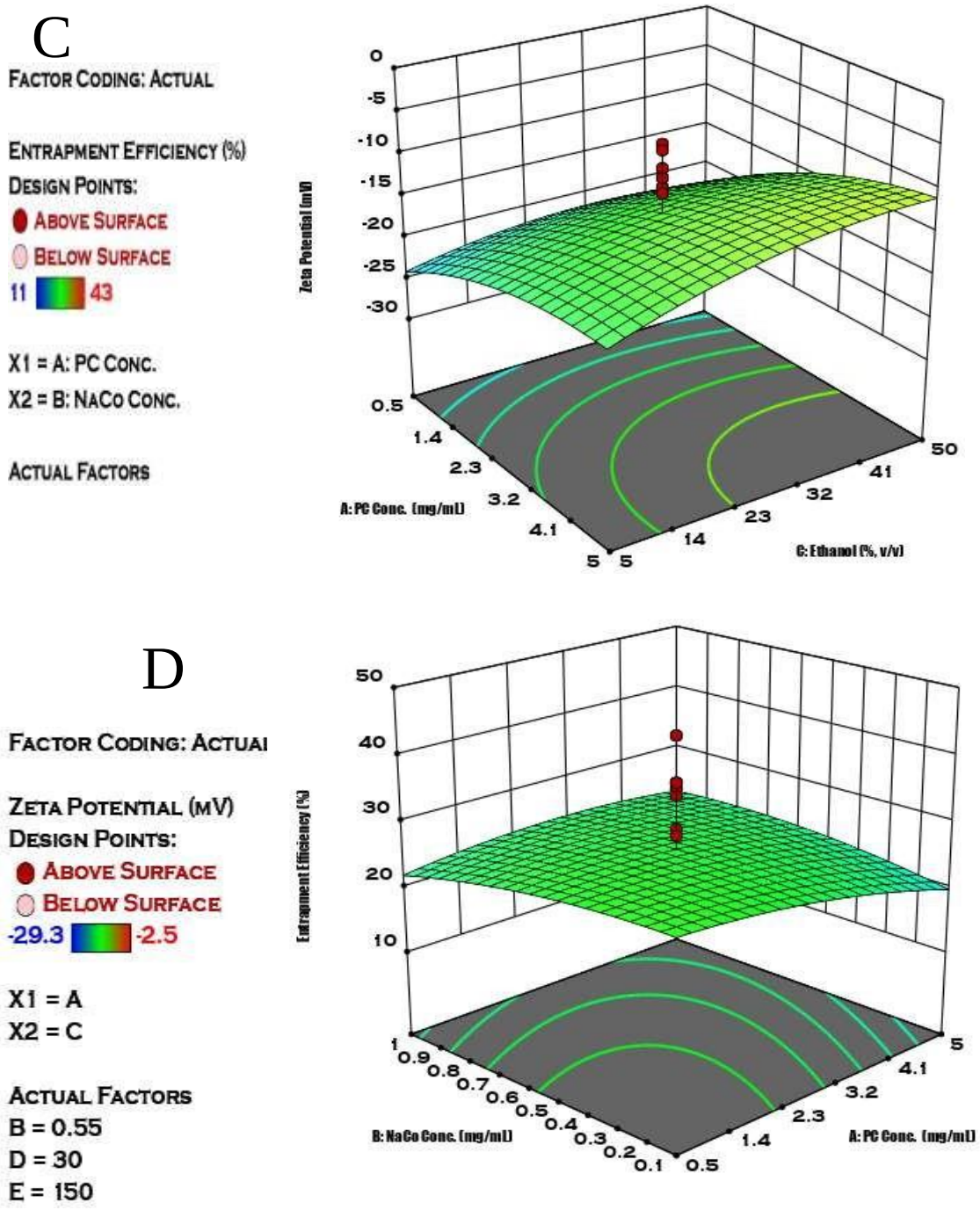


Figure 2. Response surface curves resulted from the input of dependent and independent variables.

With the above statistical presentation of the results the desired outcome was planned by giving optimization part of the design expert where vesicle size goal was taken as 100 nm (with importance factor 2), zeta potential as -29 mV (with importance factor 2), entrapment efficiency as 40 % (with importance factor 5) and vesicle deformability as 4 (with importance factor 5). The optimization tool suggested 100solutions. Among 100 solution suggested, one with desirability of 0.849 was taken as optimized formula consisting of 0.5 mg/mL of PC Conc., 0.1 mg/mL of NaCo Conc., 28.71 % v/v ethanol, temperature 48.56 ^{0}C stirring 242.5 rpm showing possibility of 151.98 nm vesicle size, -23.77 mV zeta potential, entrapment efficiency 35.82 and 3.69 vesicle deformability. The CPP were controlled as per suggested by optimization tool of design expert accepting their error ranges and readability to prepare the TEs and was taken for evaluation of vesicle size, entrapment efficiency, zeta potential and vesicle deformability. The suggested results and observed results were compared and % variation from the target value was given in table below ***Table 3***. % D_{target} (% difference of outcome with respect to target) and % $D_{Predicted}$ (% of difference of outcome with respect to predicted) 7.64 & 29.17 % for vesicle size, 6.10 & 14.56 % for zeta potential, 17.23 & 7.57 % entrapment

efficiency and 8.75 & 1.08 %, respectively. So the prediction was giving more closeness to entrapment efficiency and deformability while targets were more close to vesicle size and zetapotential.

Table 3. The prediction of optimization tool for achieving the targets of independent variables and % difference from the target and predicted outcomes.

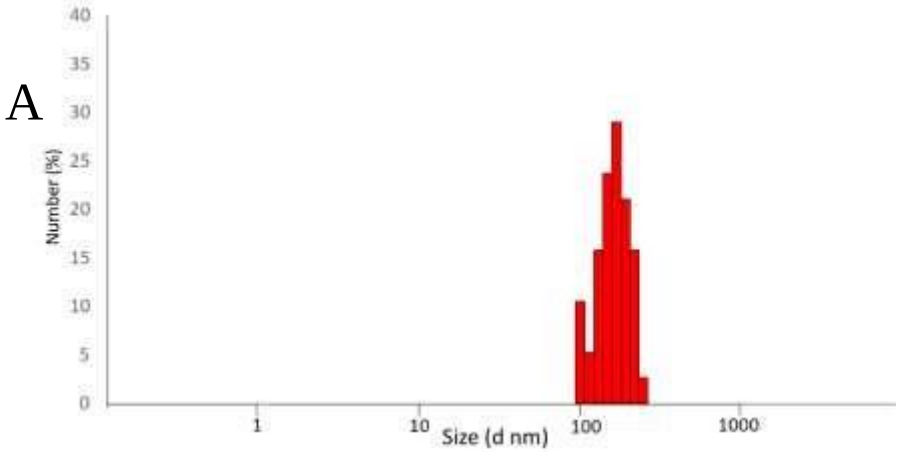
	CPPs					Independent variables			
	PC Conc mg/mL	NaCo Conc mg/mL	Ethanol % v/v	Temperature ^{0}C	Stirring Rpm	Vesicle Size nm		Zeta Potential mV	Entrapment Efficiency %	Vesicle Deformability
Target	NA					100	-29	40	4	
Prediction	0.50	0.10	28.71	48.56	242.54	151.98	-23.77	35.82	3.69	
Outcome	NA					107.64	-27.23	33.11	3.65	
% D $_{Target}$	NA					7.64	6.10	17.23	8.75	
% D $_{Predicted}$						29.17	14.56	7.57	1.08	

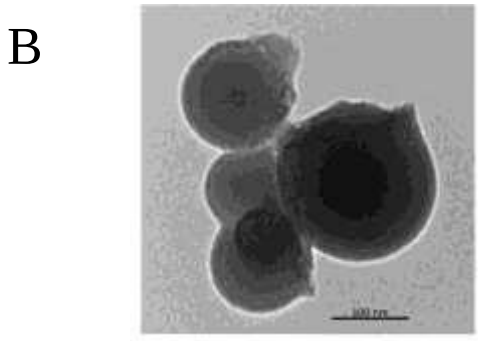
% D_{Target}: % of difference of outcome with respect to target; % D $_{Predicted}$: % of difference of outcome with respect to predicted

Physicochemical and Morphological Characterization of the Formulation

Psoralen tricyclic compound (**Figure 1**) and crystalline solid. Its solubility is reported to be 30 mg/mL in Dimethoxy sulfoxide (DMSO) and dimethyl formamide (DMF), for ionic organic solvent ethanol its solubility is reported to be 1 mg/mL (Cyamen-Chemicals 2022) while in water its solubility is reported to be 65.16 µg/mL at 25 °C (Pubchem).. Psoralen basic moiety is falling under class II of Biopharmaceutical Classification System (BCS) i.e. low solubility and high permeability (Machado, Silva et al. 2022). In order to make this compound more bioavailable solubility is the bottleneck to make it a breakthrough medication.

The dispersion of the final formulation of TE-psoralen was stable incomparison to equivalent amount of psoralen in water at 30 °C. The observed dispersion stability was further supported by the quantitative outcome of the particle's zeta potential i.e. -27.23 ± 4.11 mV. Vis a vis particle size of the TE-psoralen dispersion was 114.32 ± 17.03 nm as suggested by zeta sizer (**Figure 3A**) revealing the polydispersity index of 0.31. The zeta sizer reveals hydrodymanic size of the vesicle or particle while TEM image showed the real time images with a scale found to contain the particle size 103.44 ± 21.88 nm (**Figure 3B**).





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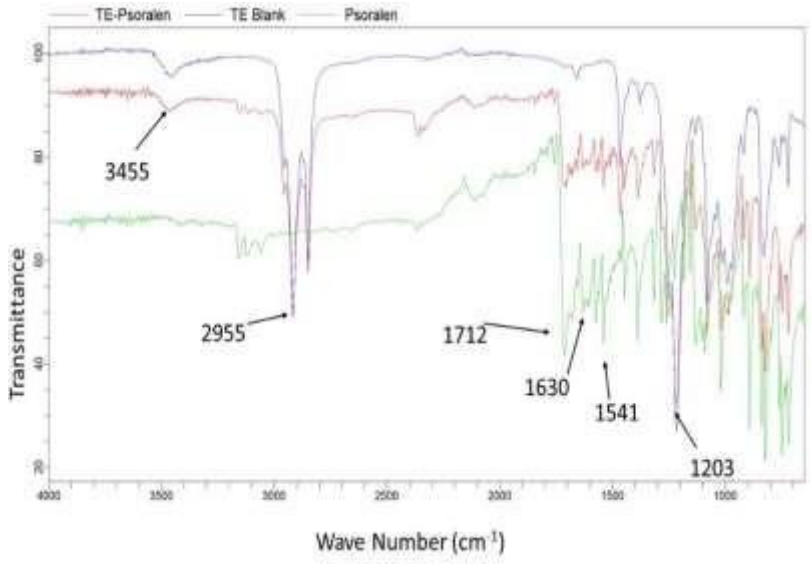

Figure 3. Characterization of the optimized batch by (A) zeta potential, (B) TEM and (C) FTIR

The FTIR analysis was conducted to authenticate possibility of interaction of chemical bonds between drug and excipients present in the formulation. **Figure 3C** showed stacked IR spectra of TE blank, Psoralen and Formulation. TE, Psoralen and TE Psoralen samples expressed the characteristic strong stretching bands at 3455 cm^{-1} as an attribute of hydroxyl moiety. The peaks at and near 2955 cm^{-1} in case of TE and TE-Psoralen formulation correspond to CH_2 stretching vibrations with long chain in phosphatidyl choline, while a significant peak of TE at 1215 cm^{-1} was also observed in TE-Psoralen attributed to the phosphate stretching band. The Psoralen and TE-Psoralen formulation both have expressed the characteristic bands of Psoralen and that of TE as well, viz 1712 cm^{-1} conforms the lactone, 1630 cm^{-1} α,β-unsaturated carbonyl (C=C-C=O) group and 1541 cm^{-1} for aromatic ring of psoralen (Sindhu et al. 2022). The FTIR band presents the existence of both the moieties present in the mixture.

Stability Study

After confirming dispersion stability the formulation stability of the optimised TE-psoralen formulation was performed by storing the formulation for 3 months at 4±2 ^{0}C refrigeration. The indicating parameters were particle size, zeta potential and leaching. The differences in the size of particle was not gradually changing, instead the fluctuation in average value could be due to the sample's non-uniformity or settlement due to long storage. There was not a significant change in zeta potential. The observable loss and consistent increase with storage duration with respect to psoralen leaching was observed after two months 4.21 ± 0.23 %. However, leaching of the psoralen may be considered out of the acceptable limit at 3rd month analysis i.e. 4.93 ± 1.43 %.

Table 4. Stability study of Optimized batch of transethosomal formulation

Months	Refrigerated Temperature (4±2 ^{0}C)		
	Particle size (nm)	Zeta potential (mv)	Leaching (%)
0	124.11 ± 8.72	-22.6	0.07 ± 0.02
1	115.25 ± 12.39	-21.2	1.86 ± 0.36
2	122.17 ± 18.10	-23.6	4.21 ± 0.23
3	119.92 ± 9.43	-19.5	6.37 ± 1.24

In-vitro drug release study

Analytical method was established with λ_{max} 246 with least interference of the impurities and retention time 6.2 minute. While **Figure 4** depicts drug release characteristics of psoralen and transethosomal formulation containing psoralen. The maximum psoralen release was observed in psoralen loaded formulation (68.32 ± 4.87 %) throughout the course of 24 h while the same of the free Psoralen was 50.32 ± 7.98 %. There was a significant change in the the release of the drug along with one more thing is to observe that with free Psoralen the saturation seems to be reached while in case of TE-Psoralen the cumulative release is still increasing even after 24 h.

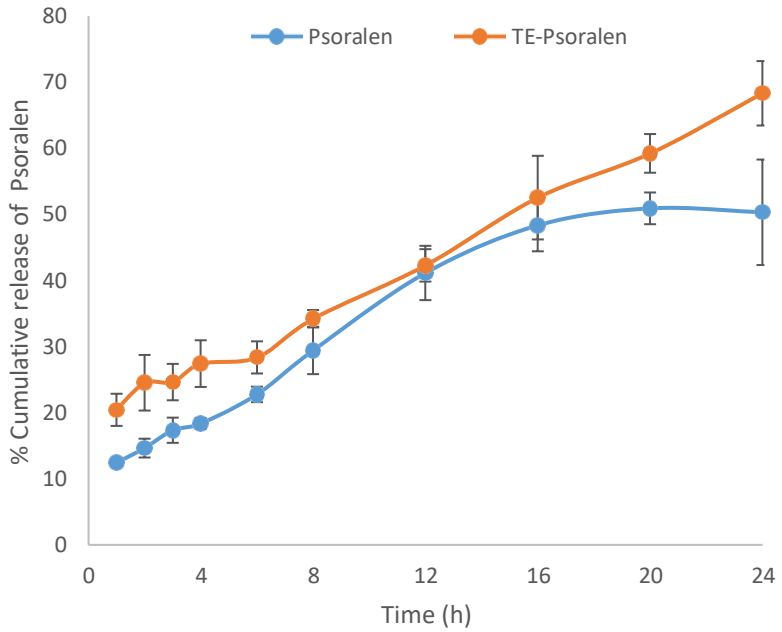

Figure 4. *In-vitro* drug release profile of Psoralen and Psoralen loaded transethosomal formulations

In vivo Study

Table 5. PASI scores observed before and after the treatment or corresponding time in case of sham and control

	PASI (Erythema)		PASI (Scaling)		PASI (Thickness)		**PASI (Overall) Sum**	
Group	Before	After	Before	After	Before	After	**Before**	**After**
Sham	0	0	0	0	0	0	**0**	**0**
Control	3	2	3	3	2	2	**8**	7
TE-Control	3	2	3	3	3	3	**9**	**8**
STD	3	1	2	0	3	1	**8**	**2**
Psoralen (1 mM)	2	2	3	2	3	2	**8**	**6**
TE-Psoralen (50 µM)	3	2	3	2	3	2	**9**	**6**
TE-Psoralen (100 µM)	3	1	3	2	3	2	**9**	**5**
TE- Psoralen (500 µM)	3	1	2	1	3	1	**8**	**3**

The PASI score for Erythema, Scaling and Thickness are presented in the **Table 5**. The sham group showed no PASI score as it has not been treated with any model disease generating drug/standard/psoralen or its formulation except shaving to the skin in order to observe the spontaneous psoriatic symptoms. Control with model disease showed the overall sum of PASI score 8 in before and after 7 in after the phase of treatments (in other groups), representing the no treatment phase has not reduced the PASI score. While TE without psoralen showed change in individual PASI negative (Erythema). Standard betnovate cream treatment show significant change in overall PASI sum (Before 8, After 2). In case of psoralin treatment 1 mM, there was moderate change PASI (Before 8, After 6). There was progressive reduction in PASI score with TE-psoralen formulation with increase in concentration with respect to psoralen.

Biochemical Analysis

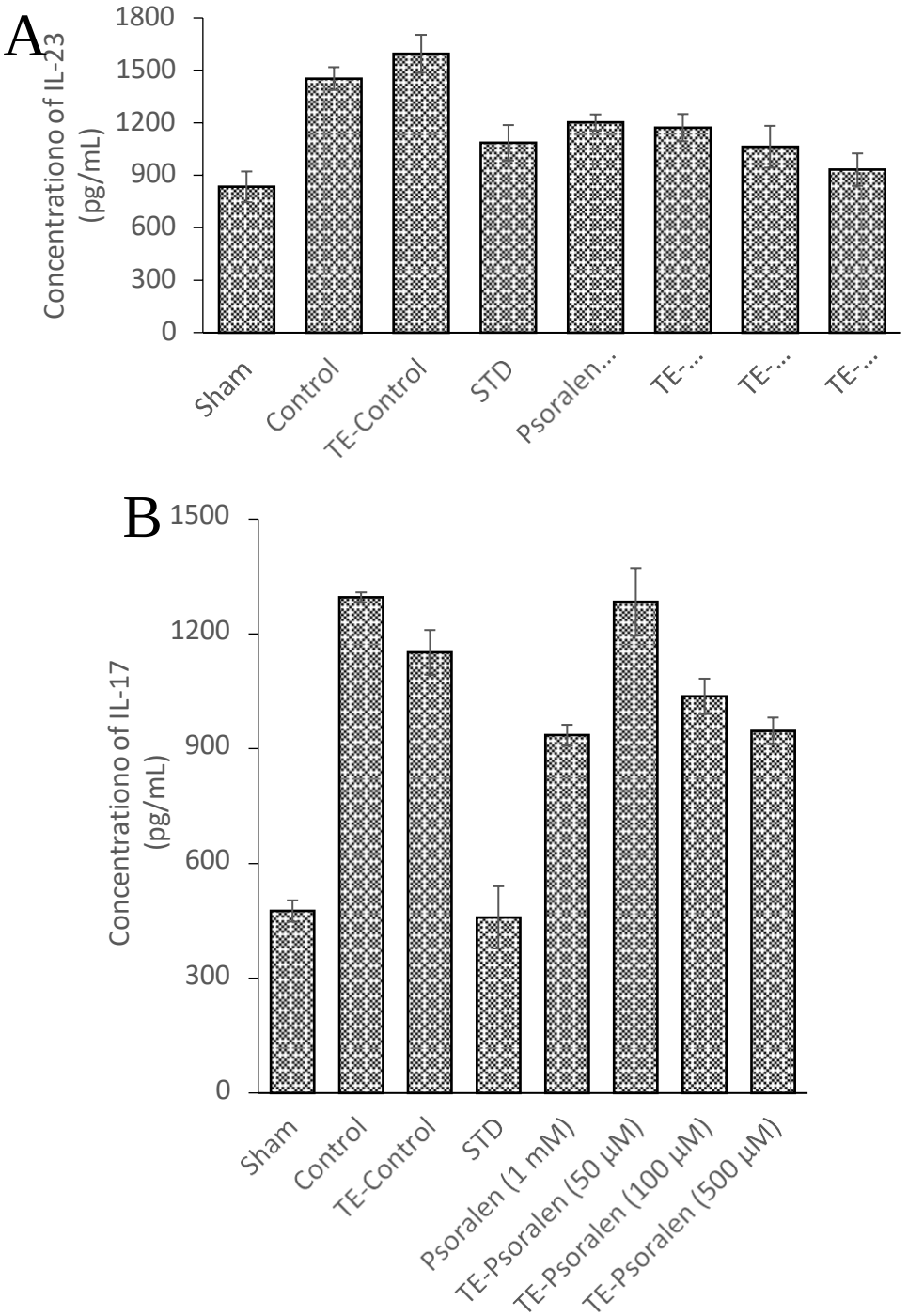

Figure 5. Expression of IL-17 and IL-23 in the skin tissue of animals in different groups.

In both the cases, i.e. IL-17 and 23, the control sample was found to have significantly high concentration of ILs and TE control insignificantly higher or lower, respectively, than the control group. Additionally, standard i.e. betamethasone showed to bring the concentration close to that of sham group value. in case of IL-23 the effectiveness of the TE-psoralen-500 µM was significantly than TE-psoralen-50 µM and psoralen- 1 mM in its expression while with respect to IL-17 the psoralen- 1 mM was statistically equivalent to the highest dose of TE-Psoralen.

Conclusion

Here this study leveraged the property of TEs to enhance the effectiveness of solubility and permeation limiting drugs. This study is also the expression of the optimization of formulation with statistical approach

to bring the state of control to any process. The outcome resulted in equation with dependent parameters' share in for particular result or attribute.

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Declaration of interest

Authors declare no conflict of interest.

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