https://doi.org/10.33472/AFJBS.6.5.2024. 7903-7919

AfricanJournalofBiological Sciences

Transethosomal Carrier of Curcumin for Improved Topical Delivery to Treat Psoriasis: Optimization, *In- Vitro*, *In-Vivo* and Stability Assessment

Gorav MONGA[1], Sandeep KUMAR[2], Rakesh KUMAR [1]*

[1] School of Pharmacy,Sharda University, Greater Noida, 201306, Uttar Pradesh, India.
[2] Department of Pharmaceutics, Nims Institute of Pharmacy, Nims University Rajasthan, Jaipur, Rajasthan, India 303121.
* Corresponding Author. E-mail: rakesh.kumar7@sharda.ac.in(R.K.); Tel. +919814996651.

Received: 0 Month 201X / Revised: 0 Month 201X / Accepted: 0 Month 201X

Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 29 May 2024
doi:*10.33472/AFJBS.6.5.2024. 7903-7919*

ABSTRACT:

Development of curcumin loaded transethosome (TE) based formulation, with statistical optimization tool to enhance and effective dissolved/bioavailable concentration of curcumin for psoriasis treatment, was envisaged in this research work. Curcumin loaded TEs were prepared by "cold method. Critical process parameters were assessed viz concentration of lipid, surfactant & ethanol; temperature and stirring. The TE formulation was planned for statistical optimization by using half fraction design with 2 blocks. The output selected were vesicle size & deformability, zeta potential, entrapment efficiency. The in vitro release profile and in vivo antipsoratic activity were performed by Franz diffusion cell and Imiquimod induced model of psoriasis in animal, respectively. Response surface and corresponding equations revealed that vesicle size the impact temperature (D) square of C (i.e. Ethanol Conc.), D and E (i.e. Stirring rate) were significant ($p<0.05$). Whereas, zeta potential was associated with A (phosphatidyl choline conc.), B (sodium cholate conc.), C, AD, BE, A^2 as significant model terms. The TE formulation was characterized with transmission electron microscope, Infrared spectroscopy. In vitro curcumin release study suggested TE-curcumin 84.21 ± 1.83 % release which was more than 2 foldw.r.t. curcumin. In vivo and biochemical studies outcomes were in accordance with enhanced release results and expressed significant higher effectiveness of TE-curcumin in comparison to same concentration curcumin. TE-Curcumin produced combined prolongs the drug contact time and releases the drug in a controlled manner, which results in improved bioavailability to the effective level for psoriasis indication.

KEYWORDS: Curcumin Transethosomes; RS; Design of Experiment; Statistical Optimization Tool; In Vitro and In Vivo Evaluation; Psoriasis

1. INTRODUCTION

Psoriasis is a disease characterized by chronic, uncomfortable leading to pain, with extreme of mental trauma & social stigma to patient and quality of life drastically drops along with depression and socioeconomic burden due to cost of treatment. In literature of medical science, it is a disease with no cure and treatment includes only ameliorating therapies. Its maximum prevalence was reported to be 11.4% in Norway thus the World Health Organization (WHO) declared it global problem in 2014 and developed a report on the same to embrace the awareness and direction to the policy makers[1].

Pathophysiology of psoriasis revealed involvement of various cytokines (TNFs, interleukins) via T-helper cells and/or Jak STAT pathways to some or more contribution extent [2, 3] Curcumin has been reported to reduce the interleukins's and TNF level in the blood [4] and also supported by many other studies. Curcumin also revealed its high effectiveness in the treating the mice model of psoriasis [5], although low response in clinical aspects (not significant in comparison to placebo) [6], significantly reducing the Psoriasis Area Severity Index (PASI) score in patients as oral adjuvant along with topical steroid [7]. Hence, the literature gives a clue to progress further research on curcumin for clinically effective and robust therapy of psoriasis.

The yellow-colored polyphenol curcumin (Cur), also known as 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione **(Figure 1)**, is taken from the rhizome of turmeric root (*Curcuma longa*) [8-10].

It has been demonstrated that curcumin has anti-inflammatory [11], anti-bacterial [12], anti-cancer [13], anti-alzheimer's[14], anti-fungal[15], and other properties[16]. Curcumin medicinal used in clinics have been limited by its low bioavailability, extremely low solubility in aqueous buffer, instability in body fluids, and fast metabolism[17, 18]. At pH 7.0 i.e. water solubility is only 0.4 µg/mL[19]. Additionally, because to their rapid hydrolytic breakdown, soluble curcumin molecules are very sensitive to physiological conditions[20]. The solubility and bioavailability of curcumin have recently been tried to improve through the use of a variety of strategies, such as the application of adjuvant molecules like piperine, quercetin, or silibinin, i.e. the use of structural analogues of curcumin; chemical complexes of curcumin with phospholipids; polysaccharides or proteins; and biological conjugates of curcumin with turmeric oil or alanine[21, 22].

In case of psoriasis the majorly focused site of action appears to be topical. Conventional topical 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[23]. Ethosomes are phosphatidylcholine (PC)-based colloidal nanosystems arranged as multilamellar vesicles that contain significant concentrations of ethanol (20–45%)[24]. Since the ethanol stabilizes the vesicles while enhancing their softness and capacity to carry lipophilic medicines, these nanosystems are more thermodynamically stable than liposomes[25]-[26]-[27]. 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[28].

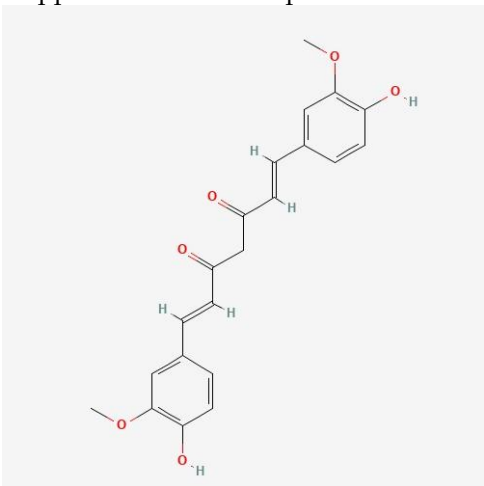


Figure 1. Chemical Structure of Curcumin. Source pubchem

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 [29]. 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[30-32]. 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[33]. 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. Due to curcumin's limited absorption, even with the dose up to 8 g/day, only traces of curcumin were found in plasma after oral dosing[34].

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 [35].

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

2. RESULTS AND DISCUSSION

2.1. Analysis of results obtained in DOE

In order to unlock the potential for superior product quality, the innovative utilization of the Design of Experiments (DOE) approach is the need of the time. By strategically manipulating independent factors, DOE empowers the creation of high-caliber products, meticulously crafted to optimize outcomes. This transformative methodology is revolutionizing the manufacturing process and elevating product to unparalleled excellence. The collective wisdom of authors, experts, and literature was harnessed to meticulously assess the parameters and their corresponding range influencing the size, zeta potential, entrapment efficiency, and deformability of TE vesicles. This collaborative effort culminated in the identification of critical factors, including PC, NaCo, ethanol concentration in the reaction and temperature, and stirring rate of the process, paving the way for targeted application of the DOE methodology. The experiment combination of the parameters suggested by DOE were carried out with respect to four outcomes or dependent variable viz. vesicle size, zeta potential, entrapment efficiency and vesicle deformability. The results were statistically evaluated with the software resulting in polynomial equations with respect to various parameters (Eqtions 3-6). These equations suggesting the magnitude of the effect of the parameter along with it suggest the agonistic or antagonistic impact based on its sign in the equation. Fit summary suggested that the all factors were of quadratic model while zeta potential and vesicle deformability were also suggested to be linear in addition. While ANOVA analysis was carried with quadratic model only.

$$\text{Vesicle Size (nm)} = 90.2 + 3.2\,A - 5.2\,B + 4.3\,C - 33.5\,D - 21.9\,E + 5.0\,AB - 5.6\,AC - 2.4\,AD + 2.0\,AE + 1.6\,BC + 0.4\,BD - 9.0\,BE + 4.75\,CD + 4.6\,CE - 3.4\,DE + 24.7\,A^2 + 8.4\,B^2 + 59.1\,C^2 + 39.2\,D^2 + 38.2\,E^2 \quad \text{(3)}$$

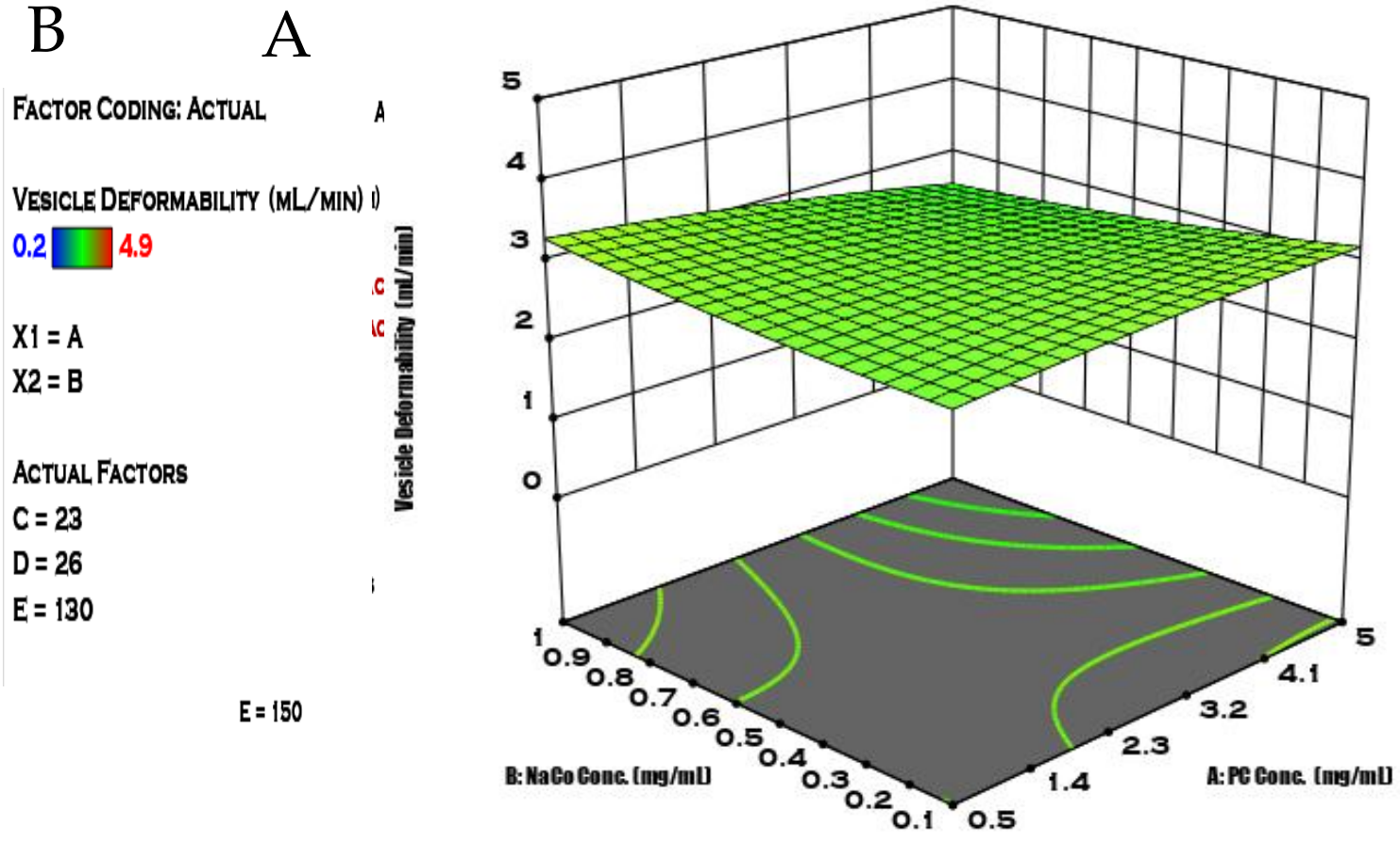
$$\text{Zeta Potential (mV)} = -14.8 + 4.7\,A + 2.2\,B + 1.9\,C - 0.7\,D + 0.3\,E + 0.4\,AB + 1.2\,AC - 3.0\,AD + 0.4\,AE + 0.3\,BC - 0.0\,BD + 2.8\,BE + 0.3\,CD + 0.5\,CE - 0.7\,DE - 2.1\,A^2 - 0.7\,B^2 - 1.8\,C^2 + 0.3\,D^2 - 0.2\,E^2 \quad \text{(4)}$$

$$\text{Entrapment Efficiency (\%)} = +25.9 - 1.8\,A - 0.7\,B - 1.7\,C + 3.5\,D + 2.6\,E + 2.1\,AB + 2.6\,AC - 2.5\,AD - 3.5\,AE - 0.7\,BC - 1.8\,BD + 0.2\,BE - 1.7\,CD - 2.8\,CE + 0.8\,DE - 1.5\,A^2 - 1.5\,B^2 + 0.4\,C^2 - 3.2\,D^2 - 2.2\,E^2 \quad \text{.....(5)}$$

$$\text{Vesicle Deformability} - 3.33 - 0.12\,A - 0.12\,B + 1.65C + 0.04\,D - 0.12\,E - 0.24\,AB + 0.09\,AC - 0.13\,AD - 0.01\,AE - 0.04\,BC - 0.09\,BD - 0.17\,BE + 0.09\,CD + 0.07\,CE - 0.01\,DE - 0.06\,A^2 + 0.07\,B^2 - 0.54\,C^2 + 0.01\,D^2 - 0.05\,E^2 \quad \text{.....(6)}$$

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 was significant. While the lack of fit was insignificant in case of zeta potential, entrapment efficiency and vesicle deformability. 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). Whereas, in the case of zeta potential A (PC Conc.), B (NaCo Conc.), C, AD, BE, A^2 were



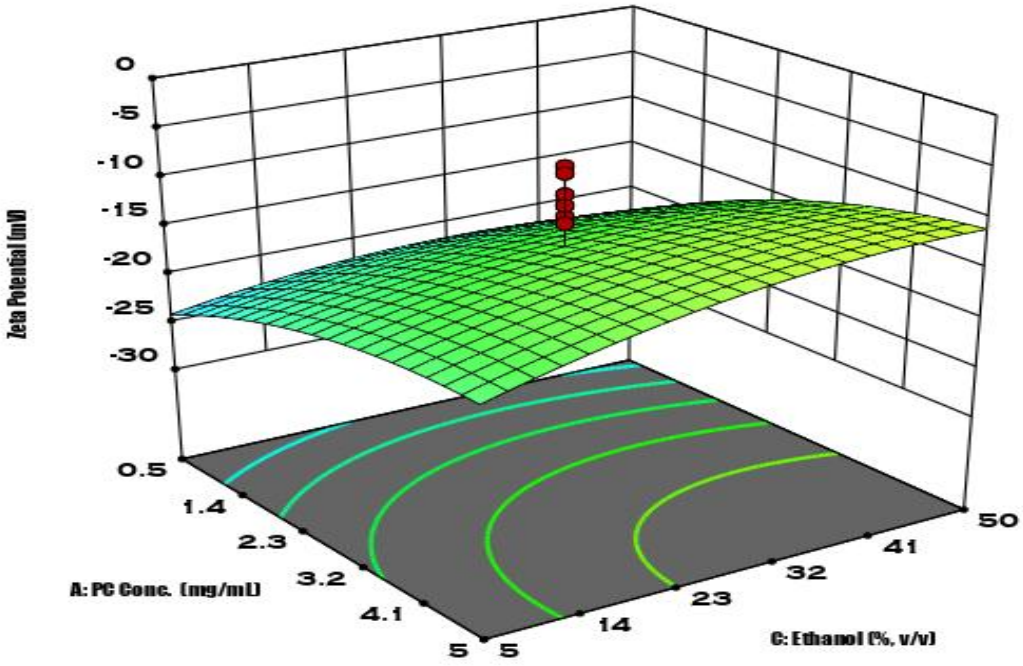
C

FACTOR CODING: ACTUAL

ENTRAPMENT EFFICIENCY (%)
DESIGN POINTS:
ABOVE SURFACE
BELOW SURFACE
11 43

X1 = A: PC CONC.
X2 = B: NACO CONC.

ACTUAL FACTORS



D

FACTOR CODING: ACTUAL

ZETA POTENTIAL (MV)
DESIGN POINTS:
ABOVE SURFACE
BELOW SURFACE
-29.3 -2.5

X1 = A

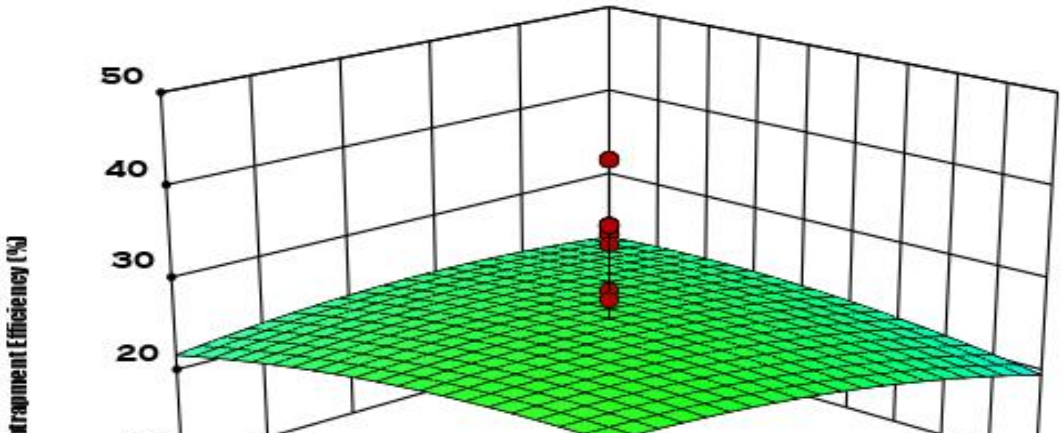


Figure 2. Response surface curves resulted from the input of dependent and independent variables.

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 0.849was 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 below ***Table 1***. % 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 zeta potential.

Table 1. 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

2.2. Physicochemical and Morphological Characterization of the Formulation

Curcumin is polyphenol compound having 3 pKa values at 7.8, 8.5 and 9.0 for 3 protons which are released at different pH. Curcumin is low soluble drug as per the classification and criteria of Biopharmaceutical Classification System (BCS). Its solubility in water and PBS is approx. 1 µg/mL and less than µg/mL respectively [36]. Hence the solubility of this compound is the limiting factor to act as therapeutic agent for wide range of indications. Its solubility in Dimethyl sulfoxide (DMSO) and ethanol has been reported to be 1 and 8.4 mg/mL respectively. Hence, formulation TE where ethanol is one of major constituent, is a justifying decision for its selection.

The optimized formulation of TE-curcumin was stable and well dispersed in comparison to curcumin in water at 30 °C. The dispersion was correlated with the zeta potential over the TE particles which was found to be -18.74 ± 2.43 mV. Zeta sizer suggested size of TE or Curcumin TE 108.42 ± 12.53 nm (**Figure 3A**) with polydispersity index of 0.25. Moreover, the vesicle size (94.21± 14.34 nm) analysis by TEM images supported the results of hydrodymanic size by Zeta sizer (**Figure 3B**).

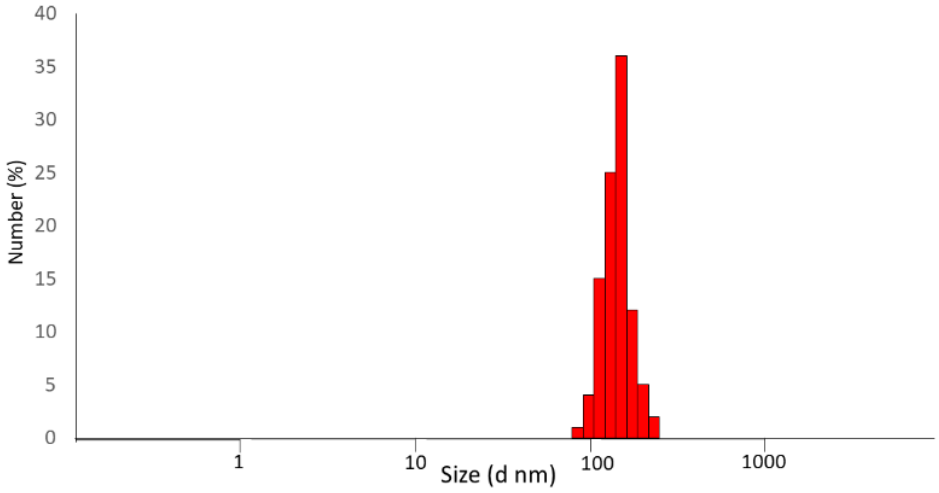
The FTIR analysis was conducted to authenticate possibility of interaction of chemical bonds between drug and excipients present in the formulation. **Figure 3C**showed stacked IR spectra of TE blank, Curcumin and Formulation.TE, Curcumin and TE Curcumin samples expressed the characteristic strong stretching bands at 3410 cm^{-1} as an attribute of hydroxyl moiety. The peaks at and near 2916 cm^{-1} in case of TE and TE-Curcumin formulation correspond to CH_2 stretching vibrations. The Curcumin and TE-Curcumin formulation both have expressed the characteristic bands of curcumin and that of TE as well, viz 1606 cm^{-1}

How to cite this article: Surname N, Surname N. Title of the manuscript. J Res Pharm. 2023; 27(X): X-XX.

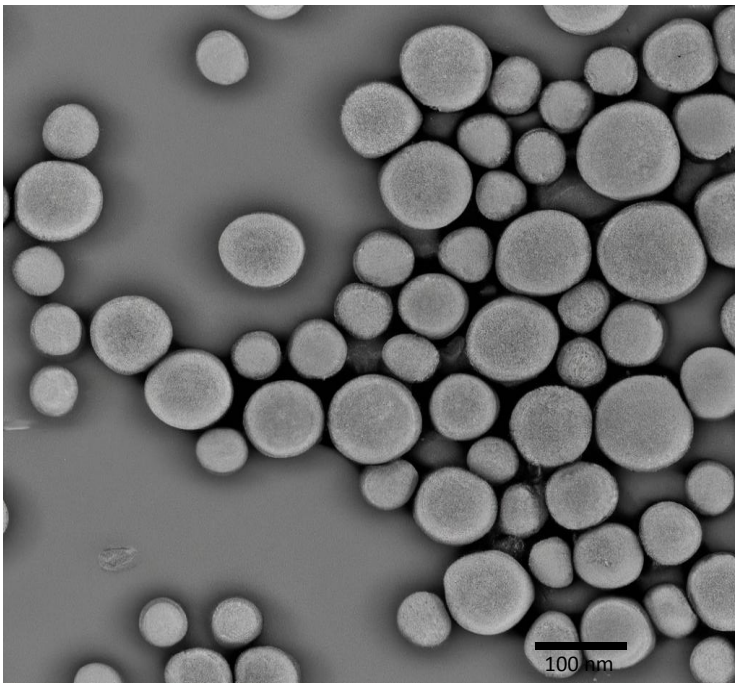
ISSN: 2630-6344
https://dx.doi.org/10.29228/jrp.2022.00

representing aromatic moiety C=C stretching, 1477 cm^{-1} representing vibration absorption with respect to carbonyl (C=O) and C=C functional groups for Curcumin. While the band at 1689 cm^{-1} represented the symmetrical stretching of the carbonyl (C=O) functional group and characteristic 1203cm^{-1} is the representation of the phosphate stretching band. The FTIR band presents the existence of both the moieties present in the mixture.

A



B



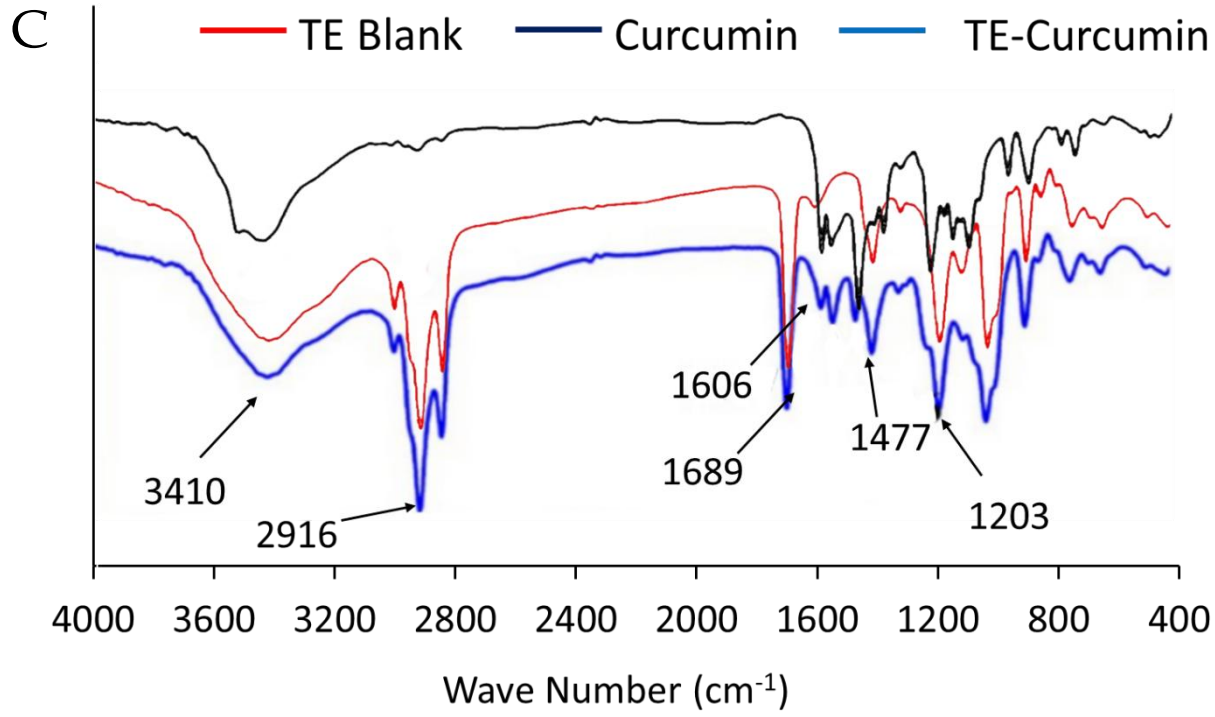


Figure 3. Characterization of the optimized batch by (A) zeta potential, (B) TEM and (C) FTIR

2.3. Stability Study

The stability of optimised TE-Curcumin formulation was evaluated by measuring hydrodymanic particle size, zeta potential and curcumin leaching percent (with respect to forced leached)at 0 day, 1, 2 and 3 months of storage at refrigerated temperatures (4±2°C). The average hydrodynamic particle size and zeta potential was remained almost same and no pattern of increase or decrease was observed. However, leaching of the curcumin may be considered out of the acceptable limit at 3rd month analysis i.e. 4.93 ± 1.43 % (**Table 2**).

Table 2. Stability study of Optimized batch of transethosomal formulation at t**emperature4±2 ^{0}C**

Months	**Particle size (nm)**	**Zeta potential (mv)**	**Leaching (%)**
0	106.32±5.25	-19.5	0.04 ± 0.01
1	108.2±8.01	-17.6	0.53 ± 0.24
2	100.76±11.65	-17.4	2.59 ± 0.23
3	105.88±6.12	-18.1	4.93 ± 1.43

2.4. *In-vitro* drug release study

Analytical method was established with λ_{max} 425 with least interference of the impurities and retention time 6.6 minute**Figure 4**. While **Figure 5** depicts drug release characteristics of Curcumin and transethosomal formulation containing Curcumin. The maximum curcumin release was observed in curcumin loaded formulation (84.21± 1.83%) throughout the course of 24 h while the same of the free curcumin was 31.32 ± 1.94 %. The release curcumin through TE-Curcumin was more than 2 folds in comparison to Free Curcumin. It is also important to note that the saturation of the release was observed at 12 h in case of Curcumin standalone while TE-Curcumin formulation showed the extended release of curcumin to the receptor compartment till the tenure of 20 h. For both the cases the kinetic of release was not fitting with zero order kinetics (R^2< 0.8) but more close to first order kinetics (R^2>0.88).

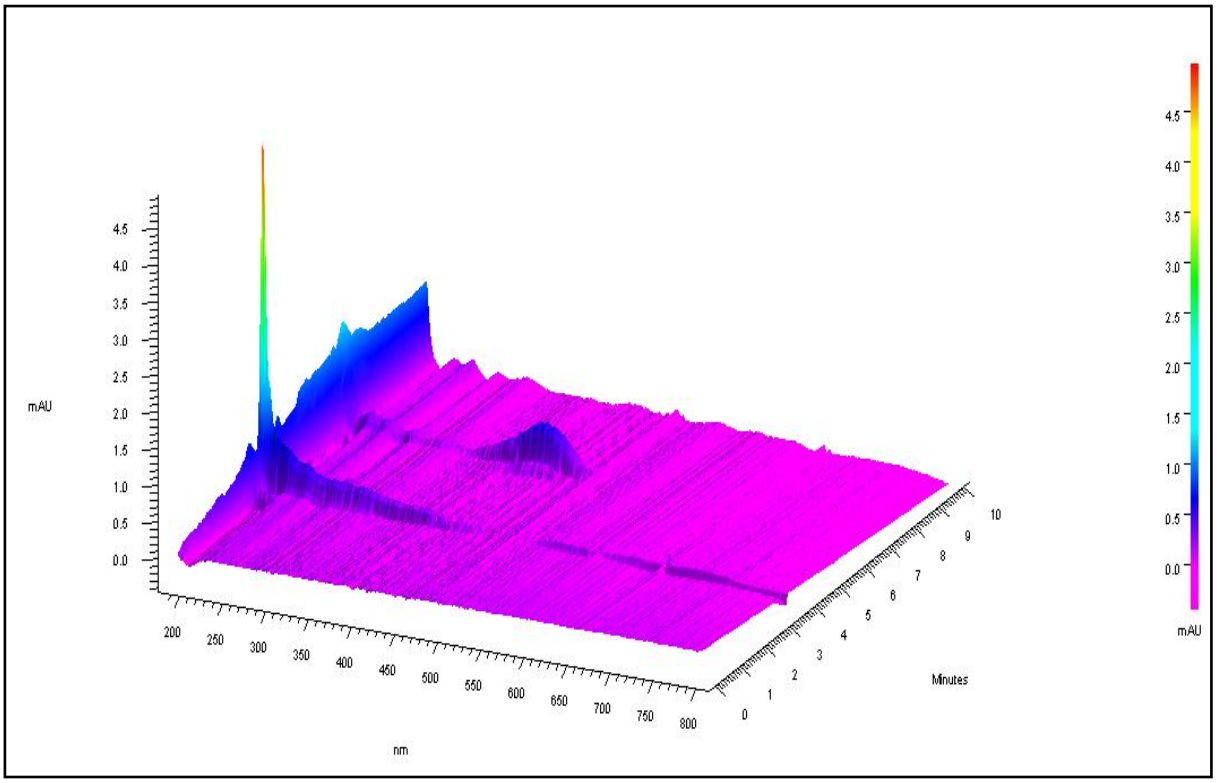


Figure 4. 3 Dimensional UV-visible scan of the chromatogram

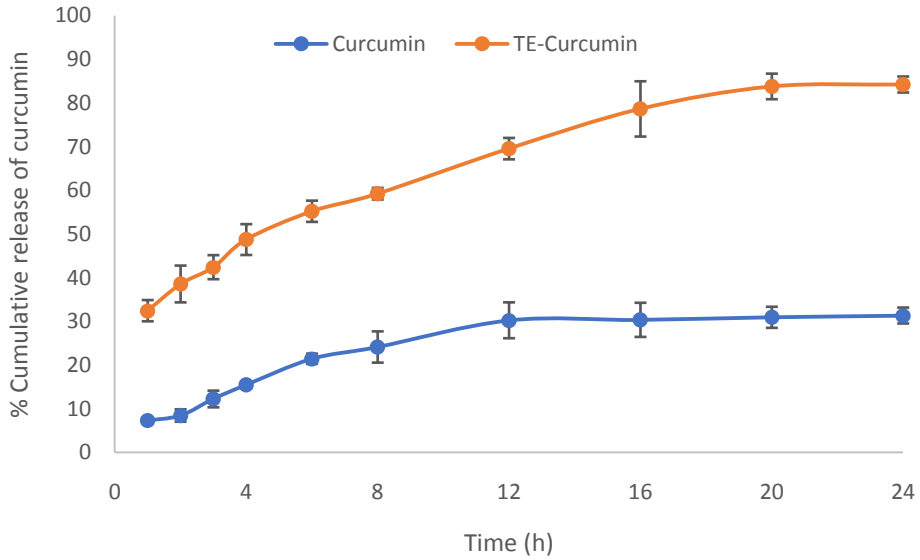


Figure 5.*In-vitro* drug release profile of Curcumin and Curcumin loaded transethosomal formulations

2.5. *In vivo* Study

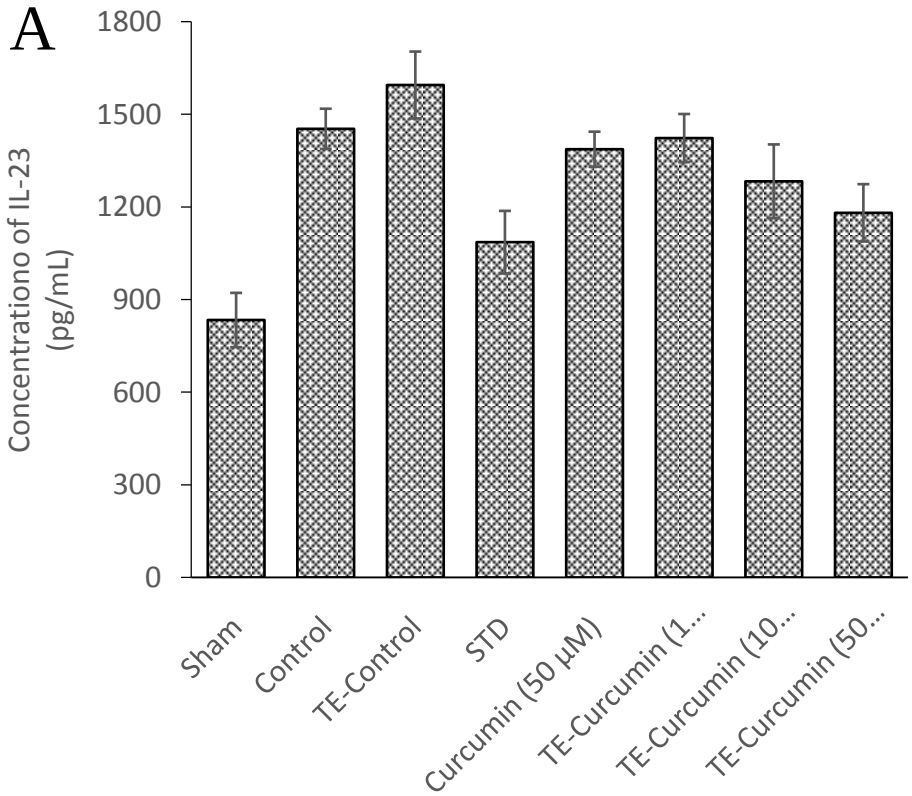
The PASI score for Erythema, Scaling and Thickness are presented in the **Table 3**.The sham group showed no PASI score as it has not been treated with any model disease generating drug/standard/curcumin or its formulation except shaving to the skin in order to observe the spontaneous psoriatic symptoms. Control with model disease showed the overall PASI score 2.3 both in before and after the phase of treatments (in other groups), representing the no treatment phase has not reduced the PASI score. While TE without curcumin showed change in individual PASI, positive (Thickness) and negative (Erythema) both but no change in PASI overall. Standard betnovate cream treatment show significant change in overall PASI (Before 2.7, After 0.7). In case of Curcumin treatment 50 mM, there was no effect on initial PASI although in this group initial PASI was higher than the control. There was progressive reduction in PASI score with TE-Curcumin formulation with increase in concentration with respect to curcumin.

Table 3. 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)**	
Group	Before	After	Before	After	Before	After	**Before**	**After**
Sham	0	0	0	0	0	0	**0**	**0**
Control	2	2	3	3	2	2	**2.3**	**2.3**
TE-Control	2	1	3	3	2	3	**2.3**	**2.3**
STD	2	0	3	1	3	1	**2.7**	**0.7**
Curcumin (50 µM)	2	2	3	3	3	3	**2.7**	**2.7**
TE-Curcumin (1 µM)	2	1	2	2	3	3	**2.3**	**2**
TE-Curcumin (10 µM)	3	1	3	2	3	2	**3**	**1.7**
TE-Curcumin (50 µM)	2	0	3	1	3	1	**2.7**	**0.7**

2.6. Biochemical Analysis

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. Effectiveness of the TE-Curcumin-50 µM was significantly effective than TE-Curcumin-1 µM and Curcumin-50 µM in expression of both ILs**(**Error! Reference source not found.**)**.



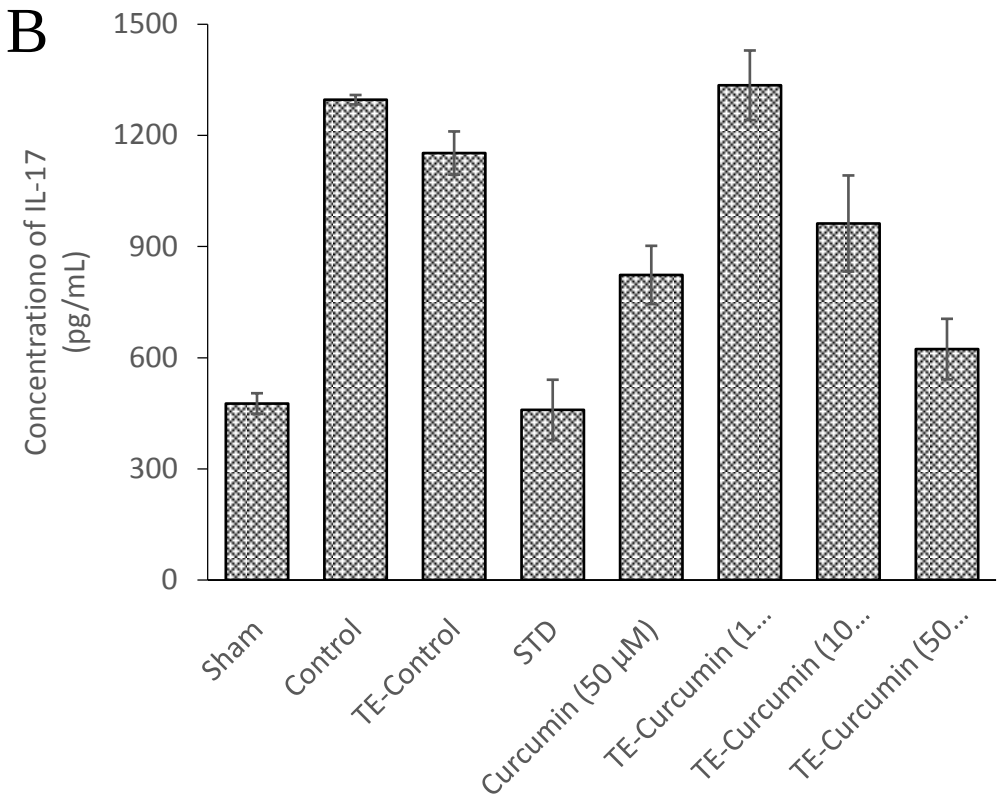


Figure 6. Expression of (A) IL-23 and (B)IL-17 in the skin tissue of animals in different groups.

3. CONCLUSION

This study revealed the utilization of the statistical tool for optimization of formulation utilizing its CPPs and their major outcome were observed. The equation and optimization study revealed the possibility of achieving the target with sufficient predictability to work upon. TE formulation showed it advantage of being deformable and enhancing solubility thus bioavailability of the solubility limiting natural product. The limiting factor for this formulation is suggested be the stability and scalability. The careful planning and a crude solution to the stability and scalability can be taken as a research to carry out further.

4. MATERIALS AND METHODS

4.1. Materials

Curcumin 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.

4.2. Preparation of curcumin loaded transethosomes

The TEs were prepared with "cold method" as given in the literature[37]. The preparation included the dissolution of curcumin 5 mg/mL, phosphatidyl choline (PC) and sodium cholate (NaCo) in ethanol followed by addition of water dropwise using a peristaltic pump to a finaly volume of 10 mL at temperature 30±4°C with continuous stirring with magnetic stirrer for 30 minutes. The amount of the all the components was taken as per the design of experiment at constant curcumin concentration.

4.3. Design of Experiment

Five critical process parameters (CPP) were determined i.e. Concentration of PC, NaCo and ethanol; Temperature and stirring during the preparation of the TEs. In Experiment design, Response Surface Method- Randomized- Central composite design was opted. All the 5 CPPs were of numeric factors, none was of categoric factor (**Table 4**). Half fraction design with 2 blocks resulted in total 36 runs, block 1- 16:8,

block 2- 10:2 (non-centre points : centre points). The Design expert proposed the set of combinations of all the factors as given in the **Table 5**

Table 4.Input for experiment design independent variables i.e. CPPs for preparation of TEs loaded with curcumin

CPP	Units	Low (-1 code)	High (+1 code)	-alpha	+alpha
PC Conc.	mg/mL	0.5	5	-1.75	7.25
NaCo Conc.	mg/mL	0.1	1	-0.35	1.45
Ethanol	%, v/v	5	50	-17.5	72.5
Temperature	°C	10	50	-10	70
Stirring	Rpm	50	250	-50	350

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

Table 5. The experiment combinations of CPPs given by design expert

Block	Run	A:PC Conc. mg/mL	B:NaCo Conc. mg/mL	C:Ethanol %, v/v	D:Temperature °C	E:Stirring 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

4.4. Drug entrapment efficiency

The each prepared formulation as per the design expert was checked for entrapment efficiency with respect to curcumin. The method involved the centrifugation at 15000 x g (Remi -03 Plus) at 10 ℃. Supernatant was checked for the curcumin content leveraging the analytical method (sub section analytical method).

The Entrapment efficiency was calculated by using the formula,

$$\%EE = \frac{Cur_T - Cur_S}{Cur_T} \times 100 \qquad (1)$$

where, EE is entrapment efficiency, Cur_T is amount of curcumin added in the reaction, Cur_S is the amount of curcumin present in the supernatant.

4.5. Vesicle deformability (VD)

The extrusion method was executed for the calculation of deformability test. TEs were extruded through a pore diameter of 50 nm of the membrane composed of polycarbonate place on filter holder (25 mm dia.). The TE suspension was given pressure of 2.5 bar at room temperature for 1 minute. The volume and vesicle size of the extruded TEs suspension was measured. VD was calculated according to equation given below

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

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 [38]

4.6. Zeta potential, Particle Size and Polydispersity Index

The charge on the particles was assessed using the Zetasizer (HORIBA ZS 100) at 25 ℃. Light scattering at the electrode voltage of 3.3 V is measured using a combination of laser Doppler velocimetry and phase analysis. The dispersion medium viscosity is 0.896 mpas.Based on light scattering principles, the mean particle size was estimated by dynamic light scattering utilising a HORIBA PS 100 Particle Sizer at 25℃ in triplicate. Before measuring, samples were diluted with distilled water. The results are shown as an average diameter of the particle size suspension (Z-average mean) vs percent sample volume, as well as the polydispersity index (PDI), which measures the homogeneity of the suspension's size distribution.

4.7. Transmission electron microscopy (TEM) evaluation

The size of the TEs was also evaluated by TEM (H-7500, Hitachi,120kV). Approximately 50 µL of samples TEs Blank and Curcumin loaded TE (CTE) were placed on a copper grid and were stained with 1% solution of phosphotungstic acid. After incubation of 5 min excess solution was blotted using filter paper followed by air drying. The images were taken at the magnification of 10k X-100k X at 80 kV.

4.8. FTIR study

FTIR Spectra of pure drug, excipients, physical mixture and optimized TE formulation were collected by using the probe (BRUKER Alpha II) FTIR Spectrophotometer. About 5 mg of the samples was inserted and analysed the spectra were obtained at 400-4000 cm^{-1}.

4.9. Chromatographic analysis of sample by using HPLC method

The concentration of curcumin was determined using reverse-phase high-performance liquid chromatography (HPLC) equipment, specifically the LC-20 AD system from Shimadzu in Japan. In this analysis, AC-18 reverse-phase column (Nucleodur C18) with dimensions of 250 mm in length, 4.6 mm in width, and a 5 µm internal diameter was utilized as the stationary phase.

The mobile phase consisted of 60% v/v acetonitrile and 40% v/v acetate buffer (5%). This mixture was flowed through the column at a rate of 1 mL/min.

Detection of the curcumin compound was accomplished using a photodiode array detector (SPDM20A; Shimadzu, Japan) with a 20 µL loop from Rheodyne. The detection wavelength used was 420 nm.

The LC Solution program served as the data station for control and data analysis.

The calibration curve generated for curcumin exhibited linearity within the concentration range of 2-10 µg/mL, with an r-squared (r2) value of 0.9939. The retention time for curcumin was determined to be 6.9 minutes. This analytical method was employed to accurately quantify curcumin levels in the samples.

4.10. Stability Study

For a period of two months, the physical stability of cucumin transethosomal formulation was assessed when they were kept in coloured glass vials with a capacity of 10 mL and refrigerated at a temperature of 4±2 °C.At the conclusion of every month, aliquot samples from each batch were taken and spectrophotometrically examined to determine the amount of curcumin that was still present. Zetasizer NanoZS (HORIBA Instrument, Japan) was used to measure changes in the size of TEsand their size distribution. The investigation also included a macroscopical visual inspection of a few transethosomal formulations for potential sedimentation and colour changes.

4.11. *In-Vitro* drug release study

The diffusion approach was used to achieve *in-vitro* drug release of transethosomal formulations. These tests were carried out using Franz diffusion cells. In the donor compartment, different quantities of transethosomal formulations with a fixed weight of 4mg were ingested. The receptor media was phosphate buffer solution pH 7.4 in the receptor compartment. The donor and receptor compartments were separated by a semi-permeable cellulose dialyzing membrane (Dialysis Membrane 12,000-14,000 MW cut-off, previously soaked in PBS pH 7.2 for 24 hours). The medium in the receptor compartment was agitated on a magnetic stirrer at 50 rpm for 8 hours at 37℃. To maintain sink conditions, 3 mL of samples were removed and replaced with new PBS solution. The obtained sampleswere diluted and analysed for drug release by HPLC method [39].

4.12. *In vivo* anti-psoriasis evaluation of Curcumin loaded Transethosomes

The experiment on animal was carried out with standard conditions and environment as suggested by the Committee for control and supervision on experimental animals (CCSEA) and with appropriate institutional ethical 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 [40].

Imiquimod based psoriasis like skin inflammation model in mice was referred from literature [41]. 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), curcumin standalone solution (50 µM) and three treatment groups 1, 10, 50 µM curcumin 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.

4.13. 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.

Acknowledgements: Authors (G.M. and R.K.) are acknowledging the School of Pharmacy, Sharda University, Greater Noida for providing the opprotunity for this research

Author contributions: Concept – G.M, R.K., S.K.; Design – G.M., R.K., S.K. Supervision – R.K.; Resources – G.M., S.K.; Materials – G.M., S.K.; Data Collection and/or Processing – G.M., S.K.; Analysis and/or Interpretation – G.M., S.K.; Literature Search – G.M.,; Writing – G.M.; Critical Reviews – G.M., S.K., R.K..

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

1. WHO. Global report on psoriasis 2016. https://www.who.int/publications/i/item/9789241565189.(Accessed on 15 Auguest 2023)

2. Abdallah MH, Elghamry HA, Khalifa NE, Khojali WM, Khafagy E-S, Shawky S, et al. Development and Optimization of Erythromycin Loaded Transethosomes Cinnamon Oil Based Emulgel for Antimicrobial Efficiency. Gels. 2023;9(2):137.

3. Armstrong AW, Read C. Pathophysiology, clinical presentation, and treatment of psoriasis: a review. Jama. 2020;323(19):1945-60. doi:10.1001/jama.2020.4006

4. Derosa G, Maffioli P, Simental-Mendía LE, Bo S, Sahebkar A. Effect of curcumin on circulating interleukin-6 concentrations: A systematic review and meta-analysis of randomized controlled trials. Pharmacol Res. 2016;111:394-404. https://doi.org/10.1016/j.phrs.2016.07.004

5. Kang D, Li B, Luo L, Jiang W, Lu Q, Rong M, et al. Curcumin shows excellent therapeutic effect on psoriasis in mouse model. Biochimie. 2016;123:73-80. https://doi.org/10.1016/j.biochi.2016.01.013

6. Kurd SK, Smith N, VanVoorhees A, Troxel AB, Badmaev V, Seykora JT, et al. Oral curcumin in the treatment of moderate to severe psoriasis vulgaris: A prospective clinical trial. J Am Acad Dermatol. 2008;58(4):625-31. https://doi.org/10.1016/j.jaad.2007.12.035

7. Antiga E, Bonciolini V, Volpi W, Del Bianco E, Caproni M. Oral curcumin (Meriva) is effective as an adjuvant treatment and is able to reduce IL-22 serum levels in patients with psoriasis vulgaris. Biomed Res Int. 2015;2015. https://doi.org/10.1155/2015/283634

8. Sun M, Su X, Ding B, He X, Liu X, Yu A, et al. Advances in nanotechnology-based delivery systems for curcumin. Nanomedicine. 2012;7(7):1085-100.

9. Yallapu MM, Jaggi M, Chauhan SC. Curcumin nanoformulations: a future nanomedicine for cancer. Drug Discov Today. 2012;17(1-2):71-80.

10. Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, et al. Chemopreventive and therapeutic effects of curcumin. Cancer Lett. 2005;223(2):181-90. https://doi.org/10.1016/j.canlet.2004.09.041

11. Peng Y, Ao M, Dong B, Jiang Y, Yu L, Chen Z, et al. Anti-inflammatory effects of curcumin in the inflammatory diseases: Status, limitations and countermeasures. Drug Des Devel Ther. 2021:4503-25. https://doi.org/10.2147/DDDT.S327378

12. Zheng D, Huang C, Huang H, Zhao Y, Khan MRU, Zhao H, et al. Antibacterial mechanism of curcumin: a review. Chem Biodivers. 2020;17(8):e2000171. https://doi.org/10.1002/cbdv.202000171

13. Tomeh MA, Hadianamrei R, Zhao X. A review of curcumin and its derivatives as anticancer agents. Int J Mol Sci. 2019;20(5):1033. https://doi.org/10.3390/ijms20051033

14. Chainoglou E, Hadjipavlou-Litina D. Curcumin in health and diseases: Alzheimer's disease and curcumin analogues, derivatives, and hybrids. Int J Mol Sci. 2020;21(6):1975. https://doi.org/10.3390/ijms21061975

15. Cheraghipour K, Ezatpour B, Masoori L, Marzban A, Sepahvand A, Rouzbahani AK, et al. Anti-Candida activity of curcumin: A systematic review. Curr Drug Disc Technol. 2021;18(3):379-90. https://doi.org/10.2174/1570163817666200518074629

16. Kant V, Gopal A, Pathak NN, Kumar P, Tandan SK, Kumar D. Antioxidant and anti-inflammatory potential of curcumin accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats. Int Immunopharmacol. 2014;20(2):322-30. https://doi.org/10.1016/j.intimp.2014.03.009

17. Yang C, Chen H, Zhao J, Pang X, Xi Y, Zhai G. Development of a folate-modified curcumin loaded micelle delivery system for cancer targeting. Colloids Surf B Biointerfaces. 2014;121:206-13. https://doi.org/10.1016/j.colsurfb.2014.05.005

18. Sun J, Bi C, Chan HM, Sun S, Zhang Q, Zheng Y. Curcumin-loaded solid lipid nanoparticles have prolonged in vitro antitumour activity, cellular uptake and improved in vivo bioavailability. Colloids Surf B Biointerfaces. 2013;111:367-75. https://doi.org/10.1016/j.colsurfb.2013.06.032

19. Li L, Xiang D, Shigdar S, Yang W, Li Q, Lin J, et al. Epithelial cell adhesion molecule aptamer functionalized PLGA-lecithin-curcumin-PEG nanoparticles for targeted drug delivery to human colorectal adenocarcinoma cells. Int J Nanomedicine. 2014:1083-96. https://doi.org/10.2147/IJN.S59779

20. Wu W, Shen J, Banerjee P, Zhou S. Water-dispersible multifunctional hybrid nanogels for combined curcumin and photothermal therapy. Biomaterials. 2011;32(2):598-609. https://doi.org/10.1016/j.biomaterials.2010.08.112

21. Naksuriya O, Okonogi S, Schiffelers RM, Hennink WE. Curcumin nanoformulations: a review of pharmaceutical properties and preclinical studies and clinical data related to cancer treatment. Biomaterials. 2014;35(10):3365-83. https://doi.org/10.1016/j.biomaterials.2013.12.090

22. Yallapu MM, Nagesh PKB, Jaggi M, Chauhan SC. Therapeutic applications of curcumin nanoformulations. AAPS J. 2015;17:1341-56. 10.1208/s12248-015-9811-z

23. Garg V, Singh H, Bimbrawh S, Kumar Singh S, Gulati M, Vaidya Y, et al. Ethosomes and transfersomes: Principles, perspectives and practices. Curr Drug Del. 2017;14(5):613-33. https://doi.org/10.2174/1567201813666160520114436

24. Natsheh H, Vettorato E, Touitou E. Ethosomes for dermal administration of natural active molecules. Curr Pharm Des. 2019;25(21):2338-48. https://doi.org/10.2174/1381612825666190716095826

25. Touitou E, Natsheh H. Topical administration of drugs incorporated in carriers containing phospholipid soft vesicles for the treatment of skin medical conditions. Pharmaceutics. 2021;13(12):2129. https://doi.org/10.3390/pharmaceutics13122129

26. Shen L-N, Zhang Y-T, Wang Q, Xu L, Feng N-P. Enhanced in vitro and in vivo skin deposition of apigenin delivered using ethosomes. Int J Pharm. 2014;460(1-2):280-8. https://doi.org/10.1016/j.ijpharm.2013.11.017

27. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. Aaps Pharmscitech. 2007;8:249-57. https://doi.org/10.1208/pt0804111

28. Mombeiny R, Tavakol S, Kazemi M, Mehdizadeh M, Hasanzadeh A, Karimi Babaahmadi M, et al. Anti-inflammatory ethosomal nanoformulation in combination with iontophoresis in chronic wound healing: An ex vivo study. IET nanobiotechnol. 2021;15(9):710-8. https://doi.org/10.1049/nbt2.12069

29. Song H, Wen J, Li H, Meng Y, Zhang Y, Zhang N, et al. Enhanced transdermal permeability and drug deposition of rheumatoid arthritis via sinomenine hydrochloride-loaded antioxidant surface transethosome. Int J Nanomedicine. 2019:3177-88. http://doi.org/10.2147/IJN.S188842

30. Verma S, Utreja P. Transethosomes of econazole nitrate for transdermal delivery: Development, in-vitro characterization, and ex-vivo assessment. Pharm Nanotechnol. 2018;6(3):171-9. https://doi.org/10.2174/2211738506666180813122102

31. Chen Z, Li B, Liu T, Wang X, Zhu Y, Wang L, et al. Evaluation of paeonol-loaded transethosomes as transdermal delivery carriers. Eur J Pharm Sci. 2017;99:240-5. https://doi.org/10.1016/j.ejps.2016.12.026

32. Albash R, Abdelbary AA, Refai H, El-Nabarawi MA. Use of transethosomes for enhancing the transdermal delivery of olmesartan medoxomil: in vitro, ex vivo, and in vivo evaluation. Int J Nanomedicine. 2019:1953-68. https://doi.org/10.2147/IJN.S196771

33. Sguizzato M, Ferrara F, Mariani P, Pepe A, Cortesi R, Huang N, et al. "Plurethosome" as vesicular system for cutaneous administration of mangiferin: Formulative study and 3D skin tissue evaluation. Pharmaceutics. 2021;13(8):1124. https://doi.org/10.3390/pharmaceutics13081124

34. Sharma S, Pawar S, Jain UK. Development and evaluation of topical gel of curcumin from different combination of polymers formulation & evaluation of herbal gel. Int J Pharm Pharm Sci. 2012;4(4):452-6.

35. Goyal P, Mishra V, Dhamija I, Kumar N, Kumar S. Immobilization of catalase on functionalized magnetic nanoparticles: a statistical approach. 3 Biotech. 2022;12(5):108.

36. Carvalho DdM, Takeuchi KP, Geraldine RM, Moura CJd, Torres MCL. Production, solubility and antioxidant activity of curcumin nanosuspension. Food Sci Technol. 2015;35:115-9.

37. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praça FG, et al. Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes. International journal of nanomedicine. 2015:5837-51.

38. Esposito E, Calderan L, Galvan A, Cappellozza E, Drechsler M, Mariani P, et al. Ex vivo evaluation of ethosomes and transethosomes applied on human skin: a comparative study. Int J Mol Sci. 2022;23(23):15112. https://doi.org/10.3390/ijms232315112

39. Kumar N, Shishu, Bansal G, Kumar S, Jana AK. Preparation and cyclodextrin assisted dissolution rate enhancement of itraconazolium dinitrate salt. Drug Dev Ind Pharm. 2013;39(2):342-51.

40. Sharma B, Dhamija I, Kumar S, Chaudhary H. In vitro and in vivo evaluation of antitumor activity of methanolic extract of Argyreia nervosa leaves on Ehrlich ascites carcinoma. ||| Bangladesh Journal of Pharmacology. 2015;10(2):399-408.

41. More NB, Sharma N, Pulivendala G, Bale S, Godugu C. Natural product topical therapy in mitigating imiquimod-induced psoriasis-like skin inflammation-underscoring the anti-psoriatic potential of Nimbolide. Indian J Pharmacol. 2021;53(4):278-85. 10.4103/ijp.IJP_591_20