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Formulation and Characterization of Curcumin Loaded Polymeric Nano Elastic Vesicular Carrier System for the Treatment of Rheumatoid Arthritis

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ABSTRACT

Background

The transdermal route of drug delivery has gained immense interest from pharmaceutical researchers. The major hurdle for the diffusion of drugs and bioactive through the transdermal route is the stratum corneum, the outermost layer of the skin. Currently, various approaches such as the physical approach, chemical approach, and delivery carriers have been used to augment the transdermal delivery of bioactive.

Materials and Method

With the use of the Box-Behnken Design, thirteen formulations were created by altering the amounts of ethanol, soy lecithin as a lipid, and the lipid: span ratio. Vesicle size, entrapment effectiveness, drug retention, drug permeability through the skin, and shape were attributes of the optimized formulation.

Result and Conclusion

The Results demonstrated the successful fabrication of the curcumin-loaded polymeric nanotransethosomes. Gels with improved CRM-TE were added, and a side-by-side analysis was done. The 180-day storage of CRM-TE gel at 5°C, 25°C, and 40°C was followed by an evaluation of its entrapment effectiveness and vesicle size. At 24 hours in the skin, CRM-TE revealed 286.4 nm vesicle size, 64.2% entrapment efficiency, 19.8% drug retention, and 71.3% drug permeation. Additionally, CRM-TE gel demonstrated the greatest qualities in terms of drug penetration, drug retention, and entrapment effectiveness CRM-TE gel showed higher stability at 5–3°C in terms of vesicle size and entrapment effectiveness gel could provide effective topical administration of curcumin.

Keywords

Transethosomes, curcumin, polymer, nanotransethosomes

INTRODUCTION

Curcuma longa, a member of the Zingiberaceae family, produces curcumin in its rhizomes [1]. It is one of the most often used medications for the treatment of viral infections, inflammatory, inflammatory, neurodegenerative, cutaneous, and metabolic problems, as well as cancer and rheumatoid arthritis [2]. By inhibiting cyclooxygenase-II and lipoxygenase and preventing nuclear factor-B from activating, it has an anti-arthritic effect. Its poor water solubility (0.0004 mg/ml at pH 7.3), which results in its low oral bioavailability, limits its utilization despite being such a promising therapeutic in the treatment of many illnesses. Due to curcumin's limited absorption (up to 8 g/day), only traces of the compound were found after oral treatment [3]. Because individuals with chronic illnesses (like arthritis) must take medication every day for the rest of their lives, the medication may accumulate in the tissues, increasing the risk of problems. So, in the current work, an effort was made to create a topical drug delivery method to boost the medication's bioavailability. The suggested delivery mechanism might bypass first-pass metabolism and result in local activity. Due to the first-pass effect, only a relatively modest amount of orally taken formulations—between 25 and 45 percent—reaches the blood circulation. The idea of gel formulations for topical treatments is developed to get around these restrictions. Despite these anticipated benefits, typical topical systems demonstrate several shortcomings in terms of deeper penetration of the skin. Polymeric elastic vesicular drug delivery systems such as liposomes (L), niosomes, transferosomes (T), and ethosomes (E) can be used to solve this issue [5]. The use of E and T might solve the issue of L's limited versatility. T include lipids and edge activators, whereas E contain lipid and ethanol, resulting in significant drug permeation [6]. A new class of vesicles called transethosomes (TE) combines the functions of T and E [5]. These combine the qualities of E and T since they contain phospholipids as a polymer, ethanol, and edge activators such as span 80, span 60, and tween 80 as a surfactant [6]. The formulation of Nano TE has been tried in the current work, and a gel for the topical administration of curcumin has been effectively created using the designed polymeric carrier system. The Box-Behnken design (BBD) has also been used to optimize formulation factors that may have an impact on the formulation characteristics of TE.

MATERIALS AND METHODS

Curcumin was procured from Dhamtech Pharma Pvt.Ltd Mumbai India. Soy Lecithin, Span 80, Ethanol, Carbopol 934, Triethanolamine were procured from Bio fusion Gwalior. All the other chemicals used for the formulation development were of analytical grade. Curcumin analysis using high-performance liquid chromatography (HPLC) Utilising reverse phase HPLC (LC-20 AD; Shimadzu, Japan), curcumin was estimated. Nucleodur C18, a C-18 reverse-phase column, is used as the stationary phase. The column measured 250 mm in length, 4.6 mm in breadth, and 5 m in internal diameter. The mobile phase was a combination of 60% v/v acetonitrile and 40% v/v acetate buffer (5%), flowing at a rate of 1 mL/min. The drug was measured at 420 nm using a photodiode array detector (SPDM20A; Shimadzu, Japan) and a 20 L loop (Rheodyne). As a data station, LC Solution software was employed. The calibrated curve was found to be linear in the 2–10 g/mL range with a r2 value of 0.9939 and a retention time of 6.9 min studies for preliminary screening to determine appropriate amounts for the formulation factors impacting the physicochemical characteristics of vesicles, preliminary

screening investigations were conducted. Different lipids were employed, including edge activators (Span 20, 60, and 80, tween 20, 40, and 80), Soy lecithin (PL 90 G, PL 70 G), and tween 20, 40, and 80. Results from the preliminary investigations showed that Soy lecithin and span 80, two edge activators, performed the best among the lipids utilized. These two were chosen as independent variables as a result. Additional formulations were created using ethanol and different concentrations of these two ingredients.

FORMULATION

Development of TE and other vesicles three alternative techniques (ethanol injection method, thin film hydration method, and fast ethanol injection method) were employed to create the vesicles. The ethanol injection approach was discovered to have the greatest outcomes out of all of them. The formula components shown in Table 1 were used to create 13 distinct formulations was represented in the table 2. Vesicles were created using a mixture of Soy lecithin (a phospholipid) and Span 80 (an edge activator). Weighed precisely, lipid, edge activator, and medication (curcumin) were dissolved in ethanol. In another tank, 50°C of phosphate buffer (pH 7.4) was maintained. The aqueous solution was injected dropwise at a rate of 1 ml/min with constant pressure with an ethanolic solution of Soy lecithin, Span 80, and medication.

DESIGN OF EXPERIMENT

The formulation factors that could affect the characteristics of vesicles were assessed in preliminary screening studies. The formulation's key characteristics included elements like the proportion of the edge activator, lipid, and ethanol. The impact of formulation and processing parameters impacting the physical qualities of TE was assessed using BBD based on the number of variables and their amounts. Critical parameters that may affect the effectiveness of entrapment, vesicle size, and skin penetration of the vesicles were considered to be the percentage of Soy Lecithin, ethanol, and Span 80 ratio. Three levels (+1, 0, and 1) of operation were used for these three parameters. All of the trials used the same medication concentration, phospholipid type, and edge activator type. The 3D response surface plot is depicted in Figure 1 showing the effect of independent variables on Entrapment efficiency, Vesicle size, and Sonication time.

Software called Design-Expert 9.0.3 from Stat-Ease in the United States was used to conduct the study. A total of 13 formulations with 2 focus points were produced during the initiative. Experiments were run in a random order to increase the predictability of the model. The independent variables and each study's design level are shown in Table 2.

Characterization of prepared formulation

The drug entrapment efficiency of the prepared formulations was studied by the mini-column centrifugation method according to the method reported by Garg et al. (2016), using the following equation [6].

Vesicle size analysis

Dynamic light spectroscopy was used on a Beckman Coulter (Delsa nano®) to analyze the size and size distribution of the vesicles. The study was carried out in triplicate, and mean values were noted.

Morphological study of optimized batch of TE4

Transmission electron microscopy (TEM) was used to assess the morphology of the optimised formulation. A drop of the diluted sample was applied to a tiny grid that was coated with copper to dry it. For negative staining, a drop of a 1% phosphotungstic acid water solution was applied after the drying process was complete. The excess solution was removed after 45 seconds, and the sample was examined under a microscope at various magnification levels [7].

Stability Study

The stability study of Optimized formulation TE4 was kept at 5±3°C, 25±3°o C, and 40±3°C for 180 days, further evaluated for entrapment efficacy and vesicle size.

Preparation of vesicular gel formulation

The produced vesicles were mixed with 1% (w/w) Carbopol 934® gel. optimised TE-based gel (TEG). Vesicular dispersion equating to 0.5% w/w of medication was added to 10% w/w of Carbopol 934® gel to create the gel. Thus, 1% weight/weight of carbopol gel made up the gel's final composition. To give the gel the correct consistency and pH (5.1), triethanolamine was added

Characterization of prepared gel

Appearance and pH The pH, clarity, colour, and presence of any particles in the produced gel were all visually assessed. Using pure water and two grams of gel, 50 mL of volume was created. We used a digital pH metre to test pH.

Measurement of viscosity

The viscosity of the prepared formulation was determined using a Brookfield viscometer at different shear rates and torque values. Spindle No. 64 was used to measure the viscosity. Measurement was done over the range of 2–100/s.

Statistical analysis

The mean and standard deviation are used to express all the results. GraphPad Prism version 7 (GraphPad Software Inc., CA, USA) was used as a statistical tool to detect potential associations using analysis of variance (ANOVA). Where p 0.05, the results were deemed significant. Utilizing the program Design-Expert 9.0.3, all formulation design and optimization studies were completed. The linear correlation plots between actual and predicted value for entrapment efficiency, vesicle size, and sonication time has been depicted in Figure 2.

RESULT AND DISCUSSION

Table 1: Selected level of independent and dependent variables used for BBD for the preparation of transethosomes

Factors/Independent variables	Name of the variables	Minimum	Maximum
X1	Soy lecithin (mg)	100	200
X2	Span 80 (mg)	25	50
X3	Ethanol (ml)	10	20
Responses/Dependent variables R1: Entrapment efficiency (%) R2: Vesicle size (nm) R3: Sonication time (RPM)	Constraints Maximum Minimum Optimum		

Table 2: Observed responses in BBD design for optimization of Tranethosomes

Run	Factor 1 A: Soy Lecithin mg	Factor 2 B: Span 80 mg	Factor 3 C: Ethanol ml	Response 1 Entrapment efficiency %	Response 2 Vesicle size nm	Response 3 Sonication time RPM
1	150	37.5	15	82	178	98
2	200	37.5	10	81	185	89
3	100	37.5	10	89	193	75
4	150	50	20	94	142	90
5	100	37.5	20	81	170	85
6	150	25	20	95	135	92
7	150	37.5	15	83	167	100
8	150	50	10	95	145	87
9	150	25	10	99	135	68
10	200	50	15	97	154	95
11	100	50	15	97	174	94
12	200	25	15	89	132	93
13	100	25	15	86	144	92
14	150	37.5	15	85	154	98
15	200	37.5	20	85	152	95

Table 3: Optimized formulation of Transethosomes by BBD

Formulation code	Amount of pure Curcumin	Factor 1 A: Soy Lecithin mg	Factor 2 B: Span 80 mg	Factor 3 C: Ethanol ml
TE1	150	200	37.5	10
TE2	150	100	37.5	10
TE3	150	150	50	20
TE4	150	100	37.5	20
TE5	150	150	25	20
TE6	150	150	37.5	15
TE7	150	150	50	10
TE8	150	150	25	10

TE9	150	200	50	15
TE10	150	100	50	15
TE11	150	200	25	15
TE12	150	100	25	15
TE13	150	200	37.5	20

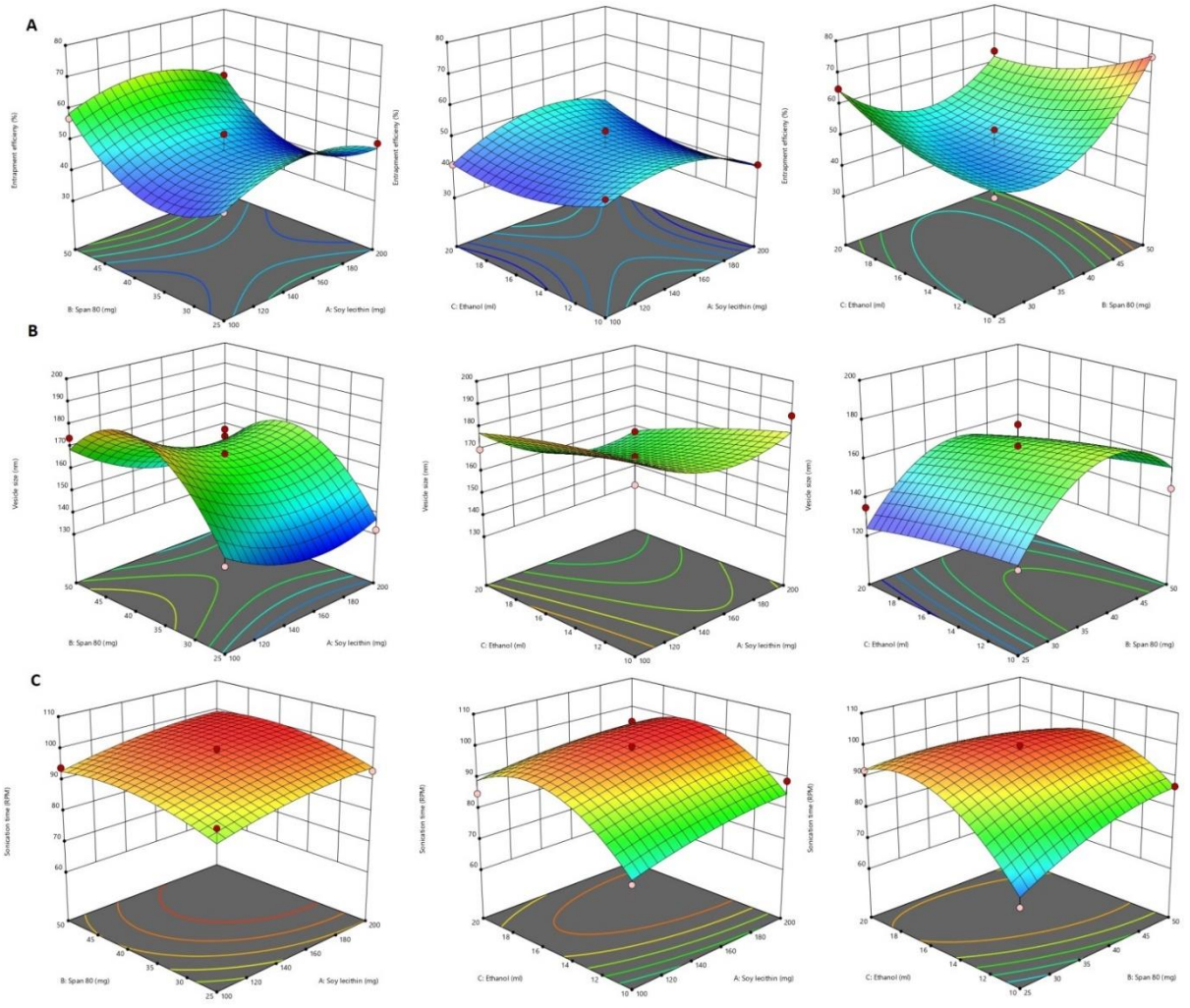

Fig. 1: 3D-response surface plot showing the effect of independent variables on (a) Entrapment efficiency, (b) Vesicle size, and (c) Sonication time

Response 1 (Y1): effect of independent variables on % entrapment efficiency

The Model F-value of 11.13 implies the model is significant. There is only a 0.81% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case B, BC, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 0.31 implies the Lack of Fit is not significant relative to the pure error. There is an 82.14% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good and the same model to fit.

Entrapment efficiency (%) = +46.47-0.1250 X_1+5.50 X_2+0.1250 X_3-0.7500 X_1X_2+3.00 X_1X_3-6.75 X_2X_3-6.83 X_1^2+12.42X_2^2+4.17X_3^2

Response 2 (Y2): effect of independent variables on vesicle size

The Model F-value of 3.10 implies the model is not significant relative to the noise. There is an 11.26% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case B^2 is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 1.17 implies the Lack of Fit is not significant relative to the pure error. There is a 49.04% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good and the same model to fit.

Vesicle size (nm) = +166.33-7.25 X_1+8.62 X_2-7.38 X_3-2.00 X_1X_2-2.50 X_1X_3-0.7500 X_2X_3+10.21 X_1^2-25.54X_2^2-1.54X_3^2

Response 3 (Y3): effect of independent variables on sonication time

The Model F-value of 5.34 implies the model is significant. There is only a 3.99% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case C, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 2.19 implies the Lack of Fit is not significant. There is an 81.32% chance that a Lack of Fit F-value this large could occur due to noise. Non- Significant lack of fit is good and the same model to fit.

Sonication time (RPM) = +98.67+3.25 X_1+2.62 X_2-5.38 X_3+0.0000 X_1X_2-1.0000 X_1X_3-5.25 X_2X_3-1.71 X_1^2-3.46X_2^2-10.96X_3^2

Table 4: Summary results of regression analysis, SD, and %CV with responses Y1, Y2, and Y3 for the quadratic model

Quadratic model	R^2	Adjusted R^2	Predicted R^2	SD	%CV
Entrapment efficiency (%)	0.9524	0.8669	0.7856	3.62	6.97
Vesicle size (nm)	0.9481	0.8748	0.7738	12.63	8.03
Sonication time (RPM)	0.9058	0.8361	0.7731	4.46	4.95

Regression Analysis

The Predicted R^2 of 0.7856 is in reasonable agreement with the Adjusted R^2 of 0.8669; i.e. the difference is less than 0.1. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 11.724 indicates an adequate signal. This model can be used to navigate the design.

The Predicted R^2 of 0.7738 is in reasonable agreement with the Adjusted R^2 of 0.8748; i.e. the difference is less than 0.1. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 16.122 indicates an adequate signal. This model can be used to navigate the design.

The Predicted R^2 of 0.7731 is in reasonable agreement with the Adjusted R^2 of 0.8361; i.e. the difference is less than 0.1. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 7.7936 indicates an adequate signal. This model can be used to navigate the design.

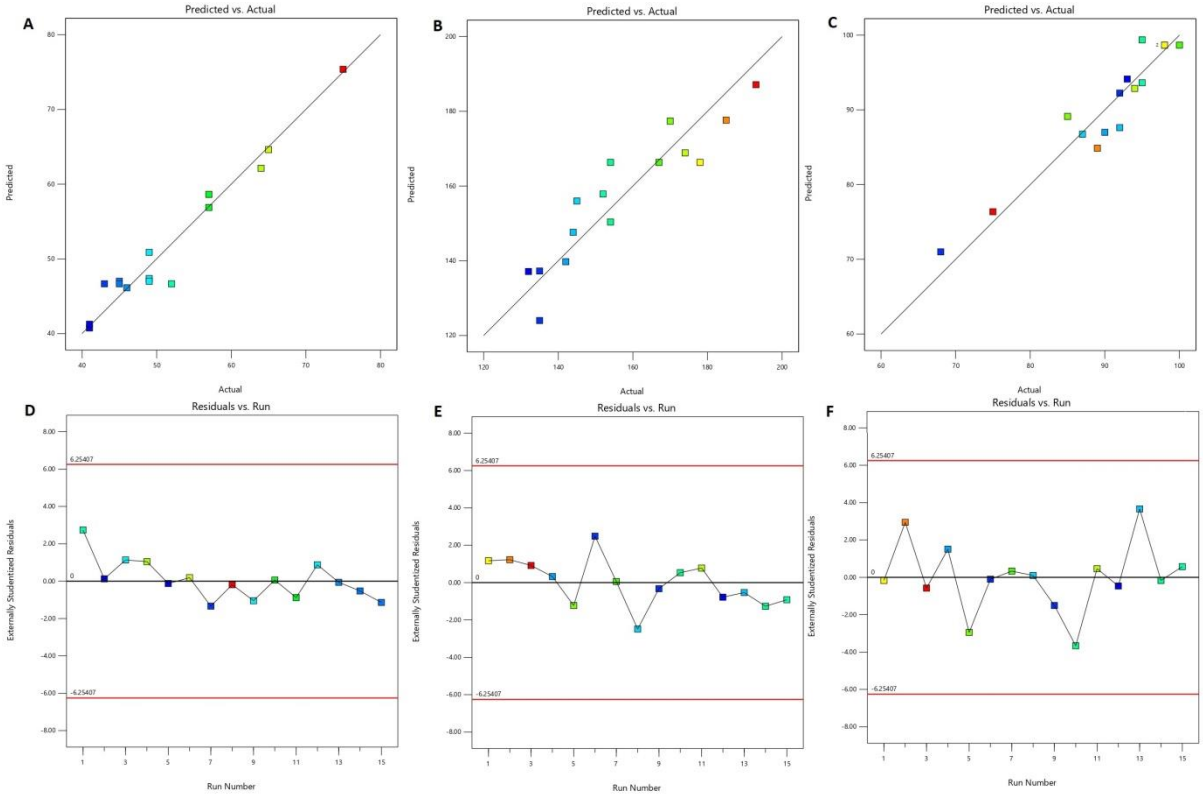

Fig. 2: Linear correlation plots (A, B, C) between actual and predicted values and the corresponding residual plots (D, E, F) for entrapment efficiency, vesicle size, and sonication time

Table 5: Mean (±SD) entrapment efficiency, particle size, and PDI of OTE, and other vesicles

Formulation Code	Mean S.D n=3 % entrapment efficiency	Vesicle size	PDI
TE	64.20±0.01	281.40±0.02	0.27±0.01
T	57.50±0.02	528.20±0.02	0.34±0.01
E	51.20±0.01	385.90±0.01	0.27±0.01
L	48.50±0.02	5735.0±0.02	1.67±0.02

Stability Studies

The stability statistics for OTE at 5°C, 25°C, and 40°C during a 180-day period are shown in Table 4. According to the results, after 180 days of storage at 5°C, there was hardly any loss of the medication that was entrapped. Nonetheless, at 252 °C, comparatively higher medication loss occurred, with the maximum loss recorded at 40°C. This may be explained by the fact that at high temperatures, vesicles lose their stiffness and combine in order to maintain the drug. At higher temperatures, there is a decrease in stiffness and an increase in fluidity. to a phase change. The formulation's vesicle size also grew at high temperatures (25°C and 40°C) which might be related to an increase in the relative rates of vesicle aggregation that was discovered At low temperatures (5–3 °C), less. Therefore, it was determined that the formulation has to be stored in a refrigerator (5–3°C) to avert any stability issues of any form.

CONCLUSION

TE was made in the current investigation using soy lecithin, Span 80, and ethanol. These were assessed for their effectiveness in entrapment, vesicle size, and drug penetration and retention after 24 hours. BBD was used to balance the amounts of Span 80, ethanol, and soy lecithin. Formulation with a substantial amount of ethanol and lipid: 95:5 span ratio was discovered to have the best qualities. the creation of TE-based Compared to other formulations, this one was proven to be more effective. L, E, and T vesicular delivery methods in terms of skin penetration skin retention, vesicle size, and entrapment effectiveness of the drug after 24 hours. For 180 days, the developed formulation was determined to be stable at 53°C. The TE containing curcumin was added to a gel made of 1% w/w Carbopol, and the rheology, drug permeation, and drug accumulation under the skin. The greatest levels of drug penetration and drug retention in the skin delayed drug release; this was shown. The release kinetics of the Hixson-Crowell model showed that the gel released the medication in an erodible way. Consequently, it may be said that the TE was discovered to be superior to other vesicular delivery techniques. and have been used with effectiveness in topical delivery in the gel-like shape. However, the in vitro and ex vivo outcomes that achieved Correlations between experiments and in vivo research are necessary.

ABBREVIATIONS

CRM-TE: Curcumin Transethosomes; TE: Transethosomes, BBD: Box Behnken Design; HPLC: High-performance liquid chromatography; ANOVA: Analysis of variance; IAEC: Institutional Animal Ethical Committee; CPCSEA: Control for purpose of control and supervision of experiments on animals; UV–VIS: Ultraviolet-visible spectroscopy.

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DECLARATION

The ex vivo skin permeation and skin irritancy studies were completed at the School of Pharmacy ITM University, Gwalior, according to the protocols permitted by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, under the reference no. 1894/PO/Re/S/16/CCSEA on the recommendations of the Institutional Animal Ethical

Committee of ITM University (Gwalior, India). All animals' requirements were completed by ITM University, Gwalior.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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