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RESEARCH ARTICLE

Optimization of Dual Phytoconstituents Cur-Ber Loaded Ultra Deformable Nanotransethosomal Hydrogel by BBD for Transdermal Delivery Enhancement: Formulation and Evaluation

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Abstract

Background: In the current investigation, the objective is to develop ultra-deformable carriers that are extremely permeable, also known as nanotransethosomes. **Aim:** The purpose of this is to improve the delivery of the dual phytoconstituents Curcumin (CUR) and Berberine (BER) for the treatment of systemic and topical pathological diseases. **Methods:** For this investigation, a Box-Behnken experimental design was undertaken. An investigation was conducted into the connection between three independent factors (x1) phospholipid, (x2) surfactant, and (x3) sonication and two dependent variables particle size (y1) and zeta potential (y2). Through the use of design analysis, the formulation that was determined to be the most effective was selected for incorporation into the Nanotransethosomal hydrogel formulation. **Results:** All the prepared CUR (Curcumin)-BER (Berberine) dual phytoconstituents-loaded TEs revealed adequate VS, ZP, and PDI. Several characteristics, including PH, *in vitro* drug release, spreadability, and viscosity, were observed in the nanotransethosomal gel formulation that had been improved. These formulations have an unpleasant taste, gastrointestinal irritation, and lower bioavailability due to the first-pass metabolism effect. In contrast to high-dose pharmaceutical treatment, continuous intravenous infusion may avoid liverwort "first pass" metabolism and maintain drug levels. **Conclusions:** CUR-BER was successfully unified into the ultra-deformable vesicle carrier and may represent a choice for administering this treatment, excluding the oral route's low bioavailability and significant negative consequences, while simultaneously improving patient compliance.

Major Findings: Dual phytoconstituent-loaded highly deformable nanotransethosomal hydrogel optimised for transdermal drug administration by Box-Behnken Design. This formulation may improve dual phytoconstituent transdermal medication delivery.

Keywords: Box-Behnken Design (BBD), Berberine, Curcumin, Nanotransethosomes, Phospholipid, Ultra Deformable

1. Introduction

Oral administration is typically used for medicine delivery due to its simplicity. These formulations have an unpleasant taste, gastrointestinal irritation, and lower bioavailability due to the first-pass metabolism effect[1,2]. In contrast to high-dose pharmaceutical treatment, continuous intravenous infusion may avoid liverwort "first pass" metabolism and maintain drug levels. However, individuals must be hospitalized and properly monitored. Transdermal drug delivery systems reduce plasma drug levels for recurring therapy, organ toxicity, early metabolism, dose-based adverse effects, gastrointestinal discomfort, and very poor bioavailability and first-pass effect[3–5]. Benefits include lower dosages, patient compliance, and regulated medication delivery[6–9]. Transdermal drugs have poorer bioavailability because the Stratum Corneum (SC), which is the highest layer of the epidermis, contains

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tight connections that prevent pharmaceuticals from entering the bloodstream[10–12]. Liposomes for topical medication delivery have opened new avenues in drug delivery research[13]. However, their irregular behavior in mixing with skin lipids, aeration out, and lingering near the skin's surface reduced skin absorbency. Thus, only topical medicine delivery was involved. Due to liposome inadequacies, transfersomes and ethosomes were created as nanocarrier systems[15,14], In the 1990s, Cevc *et al.*,[16] produced transferosomes using Surfactant (SA) or Edge Activator (EA) to provide manufactured vesicles flexibility. These systems modify shape but cannot reach the skin's deeper layers[18] In 1998, ethosomal systems with high ethanol concentration increased skin medicine absorption by mixing ethanol with lipid bilayer vesicles and increasing SC lipid saturation[19]. To combine the collaborative effects of transfersomes with ethosomes, Researcher Song *et al.*,[20] created Trans-Ethosomes (TE).

Initially invisible and readily mistaken with other conditions, rheumatoid arthritis may worsen with time[21]. Rheumatoid arthritis treatment involves painkillers, physical therapy, and sometimes surgery. Disease-Modifying Antirheumatic Medications (DMARDs) fall into three categories: traditionally manufactured (e.g., methotrexate), targeted synthetic (e.g., JAK1/2 & pan-JAK and inhibitors), and biological (e.g., IL-6R inhibitors, TNF-α inhibitors,)[22]. DMARDs may relieve rheumatoid arthritis symptoms and prevent development. However, DMARDs cause gastrointestinal toxicity and cardiovascular disease adverse effects and are expensive[23]. Some TCM formulas have anti-arthritic properties and improve symptoms. Traditional Chinese medicine may treat rheumatoid arthritis symptoms and slow its development[24].

The Zingiberaceae and Berberidaceae genus Curcumin (CUR) and Berberine (BER), a perennial plant. The most important chemical component curcumin and Berberine, has antioxidant, anti-inflammatory, anti-angiogenic, and anti-tumor properties without side effects[25]. By decreasing inflammatory reactions, reducing blood lipids, and improving blood sugar, curcumin and curcuminoids in turmeric may protect against many chronic illnesses[26]. Anti-inflammatory Curcumin and Berberine in turmeric reduce pain and swelling. Recently, curcumin and Berberine was discovered to reduce indicators of autoimmune disorders such as rheumatoid arthritis and IBD[27]. Many investigations have examined the pharmacological and clinical effects of turmeric and curcumin on rheumatoid arthritis[28].

Curcumin and Berberine reduce corticosteroids and DMARD dosages in renal transplant procedures, according to several studies[29]. This may reduce steroid side effects. This study investigates if Curcumin and Berberine TETSM carriers may be used transdermally to boost Cur-Ber's anti-inflammatory activity and pharmacokinetics.

2. Materials and Methods

2.1 Materials

Phytoconstituents Curcumin (CUR) and Berberine (BER) were acquired from Neutraherbs Pvt. ltd Mumbai. Soy phosphatidylcholine, edge activator, and ethanol were acquired from Sigma-Aldrich.

2.2 Methods

2.2.1 Experimental Design Setup

Initial screening experiments assessed formulation characteristics that may affect vesicle properties. Key formulation factors were edge activator, lipid, and ethanol. All tests used the same medication concentration, phospholipid, and edge activator. Study software used Design-Expert 13.5.5 (Stat-Ease, USA). Software with 2 focal points developed 13 formulas. Randomly ordering experiments improved model prediction. The BBD was used to create Transethosomes and study excipient effects on vesicle size and zeta potential. For DOE optimization, factors like Independent variables were (X1) phospholipids, surfactant (X2) tween 80, and (X3) sonication time. Dependent variables were vesicle size (Y1) and zeta potential (Y2). The Box–Behnken design yielded 13 experiment turns with three center points. Design expert software (13.0.5) optimized fabricated TEs. The dependent variables were vesicle size (Y1) and zeta potential (Y2). The numerical standards of R, R2, adjusted R2, projected R2 and % CV and SD of each of the four responses were recorded.

2.3 Preparation of CUR-BER loaded TETSM's

The phospholipid (soya Pc), edge activator (surfactant Tween 80 and span 20:1 ratio), and CUR were liquified in ethanol to create the organic phase for the transethosomes containing the drug (CUR-BER). The mixture was endlessly stirred at 700 rpm while maintaining a 30°C temperature. After heating the BER-loaded aqueous phase (ultrapure water) to 30°C, the aqueous phase was gradually introduced to the organic phase while being endlessly stirred at 700 rpm with a magnetic stirrer. To get the transethosomal dispersions, the mixing was done for an additional 45 minutes. Lastly, Formulated dispersions were refrigerated for examination.

2.4 Characterization of the Formulated CUR-BER Loaded TETSMs

2.4.1 Estimation of Entrapment Efficiency

To determine the EE % of curcumin and berberine inside the nano transethosomes, a centrifugation procedure was used. The nanoparticle samples were centrifuged for three hours at 10,000 rpm in a cooling centrifuge. By associating the quantity of medication administered to the nano transethosome formulation with the amount that remained in the polar phase after centrifugation, the EE % was determined. Using a UV spectrophotometer, the supernatant from the samples was carefully removed, and its wavelength was estimated to be between 372 and 280 nm. Below is a formula for figuring out EE %.

2.4.2 Zeta Potential and Size of the Vesicle Analysis

The size of the vesicle was determined using the DLS approach at 25°C with the aid of a "Zetasizer Nano ZS". Before analysis, the samples underwent a dilution of 100-fold using Milli-Q water. Restrictions like the Poly dispersity index are used to characterize the consistent small-size dispersal of vesicles for effective and stable formulations. The PDI (Poly-Dispersity Index), measured as the Z-average, was obtained by fitting the data to an autocorrelation function. The particle size and Poly-Dispersity Index (PDI) of the placebo transethosomes trial formulations were measured at 25°C (DLS) with a Malvern Zetasizer Nanoseries- ZS instrument. The instrument was equipped with a backscattered light detector operating at an angle of 173°.

2.5 Preparation of Nanotransethosomal Gels Containing Optimized TETSMs:

Several batches of hydrogel base were loaded with the optimized formula code TECB. It was then judged for spreadability, viscosity, pH, and *in vitro* drug release. TECBs were enhanced with the use of Carbopol 934 as a gelling agent. Every batch is uniform in appearance and contains the same quantity of TECB batches.

2.6 Assessment of Nanotransethosomal Gel Formulations

2.6.1 pH Evaluation

Consuming a digital pH meter, the pH of the gel was measured by fully submerging the glass electrode in the gel system to cover it. Three readings were taken throughout the measurement, and the average of the three was noted.

2.6.2 Spreadability Test

Two glass slides with standard dimensions were obtained. One slide was coated with herbal gel. The second slide was situated on the gel, with the gel crammed sandwiched between the two glass slides at a distance of 7.5 cm.. Remove any excess gel from the slides and reduce their weight. The two slides were securely affixed to a platform in a stationary manner, ensuring that only the top slides could detach due to their weight. Methodically fastened a 20 g weight onto the uppermost slide. The duration of the top slide's movement of 7.5 cm and subsequent detachment from the bottom slide due to weight was recorded. The experiment was repeated thrice in order to determine the average duration. Algorithm for determining spreadability. The formula for spreadability (S) is given by $S = m \times l/t$, where S represents the spreadability, m is the weight attached to the top slides (20 g), l is the length of the glass slide (7.5 cm), and t is the time in seconds.

2.6.3 In Vitro Drug Release Studies

Utilizing a modified dialysis bag, the *in vitro* drug release test was carried out. A beaker containing 25

ml of phosphate buffer with a pH of 7.4 was placed over the bag containing the ideal formulation gel (2gm per formulation). The receptor compartment was this buffer. Using a magnetic stirrer, the receptor compartment was mixed with the entire cell surface. Precisely 37± 1^{0}C was the temperature maintained. For a duration of 8 hours, the receptor compartment was emptied and refilled with a 1 ml sample at regular intervals. For TECB, the sample was compared to a blank using UV spectroscopy at a wavelength of 372 nm.

3. Results

3.1 Effect of Design Plan and Formulation Variables on Vesicle Size, and Zeta Potential

This study's main goal was to evaluate how well TETSMs worked to increase CUR-BER's skin penetration for the treatment of inflammatory illnesses. TETSMs' high alcohol content and surfactant qualities allow them to penetrate the stratum corneum with effectiveness[7]. A study was carried out to explore the effects of several formulation parameters on particle size (Y1) and zeta potential (Y2). These variables included the concentration of phospholipid (X1), the concentration of surfactant (X2), and the duration of sonication (X3). Table 1 provides an overview of the regression analysis findings, which were used to choose the most appropriate model for every answer.

3.2 Effect of Formulation Variables on Particle Size

From 88 to 285 nm and -16 to -68 mv, the data displayed y1, y2, responses. The significance of model Prob was confirmed by the fact that it was less than F in the ANOVA findings. The relevance of the model was indicated by the model F values of 21.19 for response y1 and 31.69 for response y2. R2 was tested for multiple regression. Responses y1 and y2 have estimated R2 values of 0.8127 and 0.9766, respectively. The modified R2 values for the variables y1 and y2 were 0.6254 and 0.9063, respectively. There was an inconsistency of less than 2 between the adjusted R2 value and the predicted R2 value. Keep in mind that a signal-to-noise ratio greater than 4 indicates adequate precision. This means the model can go into the realm of design. The fit statistics can be easily depicted in the table 2.

$y1$: Particle size = +177.07+10.72 A+8.19 B+19.09 C+15.33 AB +9.72 AC -78.90BC (1)

$y2$: Zeta potential=-19.00+2.73 A-4.11 B-6.44 C-23.68 AB+4.67 AB+ 4.67 AC-18.10 BC+0.1750 A^2-17.50B^2+1.55 C^2

By utilising the BBD design, Transethosomes were produced to examine the effect of excipients on vesicle size and zeta potential. To optimize the DOE,

Table 1. Composition of CUR-BER TETSMs formulations and the results of changed responses (mean ± SD)

Std	Run	Drug CUR-BER (mg)	A: Phospholipid (mg) X1	B: Surfactant (%) X2	Sonication Time (Min.) X3	Particle size (nm) Y1	Zeta Potential (mv) Y2
8	1	50:50	100	0.75	60	191.7	-13.5
3	2	50:50	50	1	45	123	-14.5
9	3	50:50	75	0.5	30	88.2	-37.9
13	4	50:50	75	0.75	45	155.3	-19
1	5	50:50	50	0.5	45	157.9	-60.4
10	6	50:50	75	1	30	283	-16.7
5	7	50:50	50	0.75	30	160.7	-11.7
2	8	50:50	100	0.5	45	185.4	-10.8
6	9	50:50	100	0.75	30	126	-12.4
7	10	50:50	50	0.75	60	187.5	-31.5
11	11	50:50	75	0.5	60	276.1	-17
4	12	50:50	100	1	45	211.8	-59.6
12	13	50:50	75	1	60	155.3	-68.2

the following independent variables were used: X1, phospholipids, X2, and X3, surfactant tween 80, and sonication time. A vesicle's size (Y1) and its zeta potential (Y2) served as the dependent variables in this study. The manufactured TEs were fine-tuned with the help of design expert software (13.5.5). The study was based on both the vesicle size (Y1) and the zeta potential (Y2). R, R2, corrected R2, projected R2, SD, and % CV were the numerical values of the four responses.

There is a mismatch more than 0.2 between the Adjusted R^2 value of 0.6254 and the Predicted R^2 value of 0.0092. Based on this finding, it seems like there's a big block effect or something is wrong with your model or data. A sufficient level of accuracy measures the signal-to-noise ratio. Optimal ratio is greater than 4. It appears that the signal is adequate, as the ratio is 7.791. When it comes to guiding the design space, this paradigm works. In the below table 3 (Figures 1 and 2) the quadratic model of ANOVA has been illustrated.

The F-value of 13.90 for the model suggests that it is statistically significant. The occurrence of noise has a chance of 2.65 % of resulting in this notable F-value. Model terms with P-values less than 0.0500 are considered statistically substantial. AB, BC, and B^2 are important variables in the model. Terms with values greater than 0.1000 are considered to be statistically insignificant. Model reduction may improve your model if it contains many insignificant terms (excluding hierarchical words). The zeta potential in all TE formulations ranges from -11.7mV to -60.5mV.

Final Equation in the Terms of Coded - Factors

Zeta potential = -19.00+2.73 A-4.11 B-6.44 C-23.68 AB+4.67 AB+ 4.67 AC-18.10 BC+0.1750 A^2-17.50B^2+1.55 C^2

The equation, articulated in terms of coded factors, enables the prediction of the reaction for certain amounts of each element. The evasion coding assigns a value of +1 to represent high levels of the components, and a value of -1 to represent low levels. The coded equation is valuable for determining the comparative influence of the elements by comparing the coefficients of the factors. The polynomial equation revealed that phospholipids, ethanol, and surfactant hurt zeta potential. The presence of a high quantity of ethanol in TE vesicles resulted in a negative zeta potential.

Table 2. Fit statistics from Box Behnken design

Std. Dev.	34.28	**R^2**	0.8127
Mean	177.07	**Adjusted R^2**	0.6254
C.V. %	19.36	**Predicted R^2**	0.0092
		Adequate Precision	7.7914

3.3 Optimised Formula for the Nano Transethosomes Formulation

Nanotransethosomes are made using optimized formula TE8. Later, this formulation was chosen for drug inclusion and coded as TE CB and TEB. The optimized formulation of nanotransethosome has been depicted in Table 4.

Table 3. ANOVA for Quadratic model response 2: Zeta potential

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5171.15	9	574.57	13.90	0.0265	significant
A-Phospholipid	59.40	1	59.40	1.44	0.3167	
B-Surfactant	135.30	1	135.30	3.27	0.1682	
C-Sonication time	331.53	1	331.53	8.02	0.0661	
AB	2242.02	1	2242.02	54.22	0.0052	
AC	87.42	1	87.42	2.11	0.2419	
BC	1310.44	1	1310.44	31.69	0.0111	
A^2	0.0700	1	0.0700	0.0017	0.9698	
B^2	700.00	1	700.00	16.93	0.0260	
C^2	5.49	1	5.49	0.1328	0.7397	
Residual	124.04	3	41.35			
Cor Total	5295.19	12				

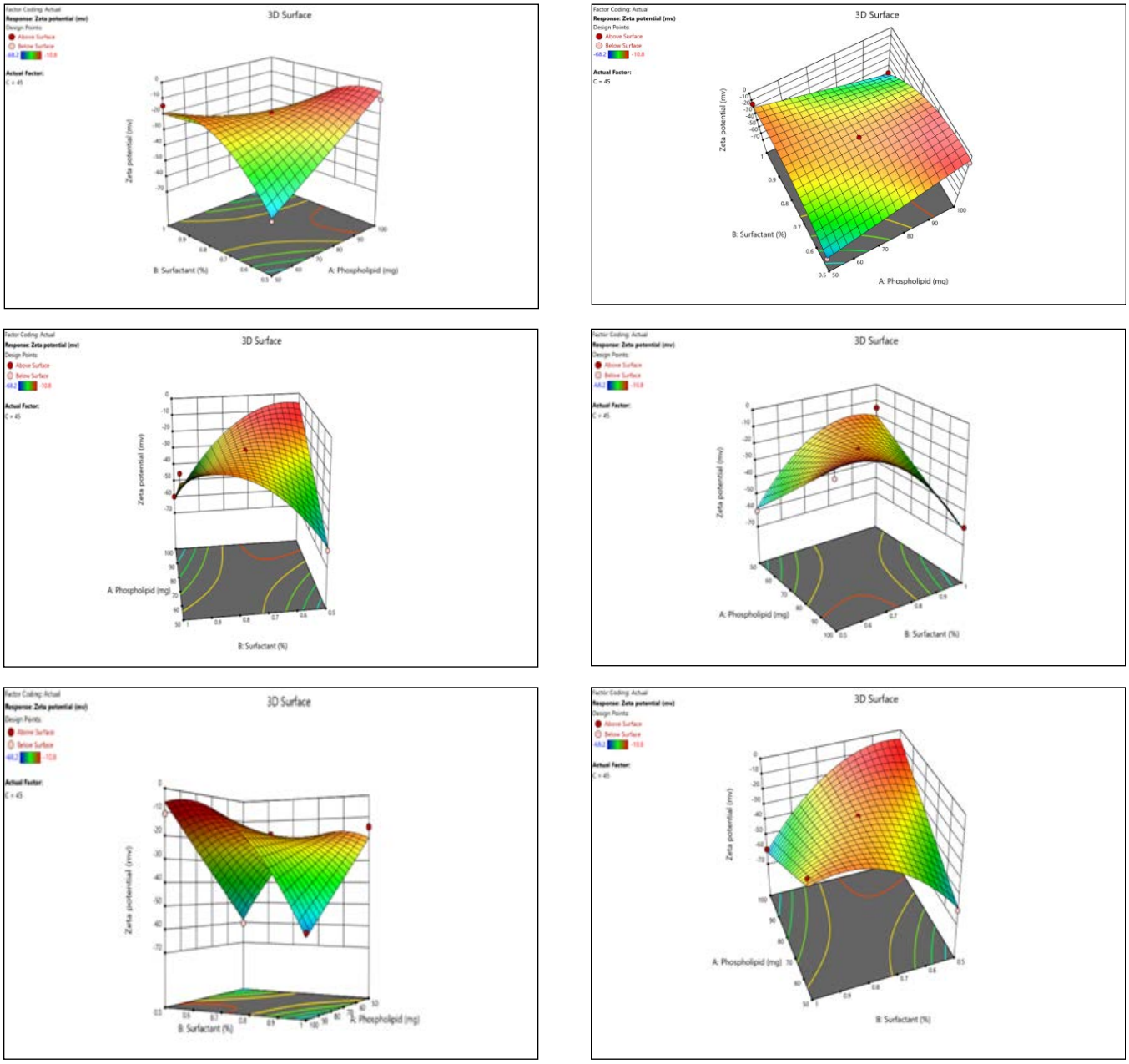


Figure 1. Show the zeta potential's three-dimensional graph using dependent variables.

3.4 *In Vitro* Release of Curcumin and Berberine from Optimized TETSMs Containing Gel

Modified dialysis bag released drugs *in vitro*. The receptor compartment of a beaker with 25 ml of 7.4 pH phosphate buffer contained a bag of optimal formulation gel (2gm apiece). All cell surfaces were in touch with the receptor compartment, which was agitated with a magnetic stirrer at 37± 1^0 C. Over 8 hours, 1 cc of receptor compartment was sampled every hour and replenished. TECB and TEBG were evaluated at wavelength against blank using UV spectroscopy (372 and 280 nm, respectively). The release profile of CUR-BER and Berberine is illustrated in Figure 3.

Different kinetic models were utilized to compute regression coefficients (R2) from *in vitro* release investigations. All studied TECBG formulations released medication using a Korsmeyer kinetic release model with first-order kinetics and a correlation coefficient larger than zero.Korsmeyer–Peppas release

Table 4. Optimised formula for nano transethosomes formulation (loaded combination of curcumin and berberine)

S. No.	Formulation Code	Drug amt (mg)	Phospholipid (Soya PC) (mg)	Edge Activator (tween 80 : span 20) (%)	Ethanol 30% (W/V) (ml)	Distilled water (ml)	Sonication time
1	TE 8 (placebo)	-	90.509	0.652	50	30	55
2	TE CB	50:50	90.509	0.652	50	30	55
3	TE B	50	90.509	0.652	50	30	55

Note: CB indicate curcumin and berberine; TE B indicate Berberine

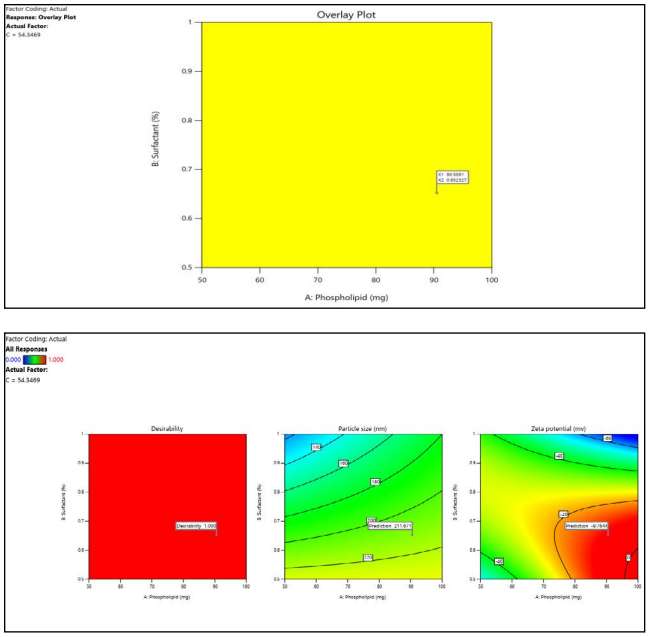


Figure 2. Represents the overlay graph and contour plots with the desirability, particle size, and zeta potential.

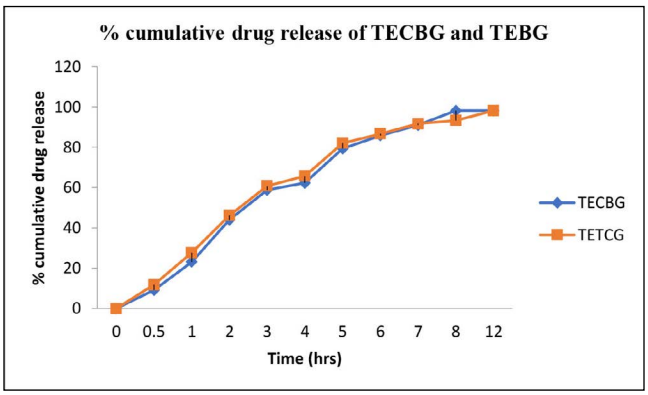


Figure 3. % cumulative drug release of TECBG and TEBG.

exponent data demonstrated a non-Fickian anomalous release with regression r^2 = 0.999 of optimized formulation TECBG.

Table 5. Characterization of nanotransethosomal hydrogel

Formula	pH	Viscosity cp	Spreadability gm.cm/s
CUR-BER Loaded TEG	7.1±0.02	5449±0.054	24±0.07

3.5 Morphological Examination of CUR-BER-Loaded TETSMs

The transmission electron picture of the optimal formula is shown in Figure 4. Spherical vesicles with enormous watery centers are shown. Smooth, well-defined, non-aggregated vesicles indicate stability. The picture matches size analysis data.

3.6 Characterization of Gel Formulations

CUR-BER gels were studied for pH, viscosity, and spreadability. Table 5 displays characterization data. According to the literature, all formulations had pH around 7.1, which is dermatologically acceptable[38]. All formulae were consistent.

Spreadability helps patients agree and apply gel uniformly. An appropriate gel spreads quickly and easily on the skin. Spreadability shows that the gels spread easily with a sheer minimum and have good adhesion.

4. Discussion

The present work sought to assess how formulation variables and design plan affected vesicle size and zeta potential of CUR-BER-loaded Transethosomes (TETSMs). Considering phospholipid content (X1), surfactant concentration (X2), and sonication time (X3) as independent variables, the Box-Behnken Design (BBD) helped to optimize formulation by means of vesicle size (Y1) and zeta potential (Y2).

4.1 Variations in Formulating Variables: Effect on Vesicle Size

The results showed a clear effect of formulation factors since vesicle size ranged from 88.2 nm to 283 nm. Vesicle size was found to be much influenced by all three independent variables—phospholipid content, surfactant concentration, and sonication time—by the regression analysis and ANOVA[39]. The positive coefficients in the equation implied that whilst an increase in surfactant concentration reduced vesicle size, an increase in phospholipid concentration and sonication time resulted in larger vesicles. This is consistent with the accepted theory that surfactants can improve vesicle stability and lower size by avoiding aggregation, whereas increased size. phospholipid levels can encourage vesicle fusion, hence increasing the total particle size. Using R^2, adjusted R^2, and projected R^2 values, one might assess the model's statistical fit[40]. The model effectively caught the variety in responses, according to the high R^2 values—0.8127 for vesicle size and 0.9766 for zeta potential. But the notable variation in adjusted R^2 (0.6254) and anticipated R^2 (0.0092) for vesicle size revealed a likely model deficit, therefore pointing out a block effect or data discrepancy. The strong signal-to-noise ratio of the model was verified by the suitable precision value (7.7914), which also qualified for design space optimization.

The *in-vitro* drug release tests revealed for CUR-BER-loaded transethosomes a continuous release profile. With a strong correlation coefficient (R^2 = 0. 999), the drug release displayed non-Fickian anomalous diffusion according to a Korsmeyer-Peppas kinetic model.[41] This implies that mechanisms of diffusion and erosion both helped to release drugs, therefore guaranteeing continuous medication availability at the target site. The last nanotransethosomal hydrogel formulation showed good physicochemical characteristics: an adequate pH (7.1 ± 0.02), viscosity (5449 ± 0.054 cp), and great spreadability (24 ± 0.07 gm.cm/s). These features guarantee efficient medication absorption via the skin, patient compliance, and simplicity of application.

5. Conclusion

The current study shows that the incorporation of dual phytoconstituents CUR-BER TETSMs and transdermal administration led to a rise in the effectiveness of the treatment for both topical and systemic pathological diseases. Additionally, the pharmacokinetics of curcumin and berberine were improved, which resulted in an improvement in the efficacy of the therapy. By harnessing the advantages of both modifying the route of administration and the insertion of dual phytoconstituents in nanotransethosomal gel form, these results bring to light a method that may be used to reduce the negative effects that are caused by synthetic medications. It is anticipated that this would result in improved treatment of inflammatory disorders and rheumatoid arthritis, as well as a reduction in the adverse effects associated with the use of synthetic medications.

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7. Author Contribution

Vijeta Bhattacharya: Conceptualisation, methodology, investigation, data analysis, manuscript writing, and editing. M. Alagusundaram: Supervision, validation, resources, and critical review of the manuscript. All authors have read and agreed to the published version of the manuscript.

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