JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

RESEARCH ARTICLE

Formulation Development and Optimization of Crisaborole-Loaded Deformable Transferosomes for the Topical Management of Psoriasis

Shailendra Patel[1], Arun Patel[2], Swarnali Das Paul*[1]

[1] *Department of Pharmacy, Shri Shankaracharya Professional University, Durg, Chhattisgarh, India*
[2] *Faculty of Pharmacy, Shri Ram Group of Institutions, Jabalpur, Madhya Pradesh, India*

Publication history: Received on 9th February 2026; Revised on 19th March 2026; Accepted on 21st March 2026

Article DOI: 10.69613/13j7yt34

Abstract: Psoriasis is a chronic inflammatory dermatosis characterized by keratinocyte hyperproliferation and a severely compromised epidermal barrier, which together restrict the clinical efficacy of conventional topical therapeutics. This study details the development, optimization, and physiochemical characterization of ultra-deformable transferosomes loaded with the selective phosphodiesterase-4 inhibitor, crisaborole, to enhance localized dermal delivery. Prepared via thin-film hydration using soya lecithin and polysorbate 80 (Tween 80), the vesicular carriers were statistically optimized using a three-factor, three-level Box–Behnken experimental design. The optimized transferosomal dispersion was subsequently incorporated into structural gel and ointment matrices to evaluate their clinical applicability. The optimized vesicular suspension exhibited an average hydrodynamic diameter of 167.58 nm, a stable zeta potential of -29.6 mV, and a high drug entrapment efficiency of 94.07%. Morphological analysis highlighted dehydration-induced crystallization phenomena typical of saline-buffered vesicular suspensions under high-vacuum electron microscopy. Rheological and physical profiling of the developed transferosomal gel (TFSOF 3) and ointment (TFSOF 3) confirmed appropriate spreadability, skin-compatible pH, and sustained-release profiles, achieving cumulative drug releases of 90.49% and 95.46% over extended periods, respectively. Mathematical modeling of the release profiles indicated a primary adherence to zero-order and Higuchi diffusion kinetics. *In vivo* dermatological tolerability and safety studies on Wistar rats confirmed the absence of skin irritation, while accelerated stability testing under International Council for Harmonisation guidelines established robust physical and chemical integrity over three months. These findings indicate that ultra-deformable transferosomes represent a highly efficient and safe topical nanocarrier platform for the targeted, sustained delivery of crisaborole in the management of plaque psoriasis.

Keywords: Crisaborole; Ultra-deformable Vesicles; Psoriasis; Localized Dermal Delivery; Hydrophilic-Lipophilic Balance; Controlled Drug Release.

1. Introduction

Topical pharmacotherapy plays an important role for managing mild-to-moderate plaque psoriasis and serves as a vital supportive measure alongside systemic interventions in severe manifestations of the disease [1]. However, achieving high patient adherence and therapeutic success with conventional topical formulations remains a significant clinical challenge. Large-scale patient surveys reveal that approximately 70% of individuals undergoing topical treatment for psoriasis report moderate-to-high levels of dissatisfaction [2, 3]. This therapeutic failure is primarily driven by poor dermal penetration of active molecules through the hyperkeratotic psoriatic plaque, rapid clearance from the tissue, and localized adverse effects that compromise patient compliance [4]. Modern developments in lipid-based nanotechnology have introduced novel delivery platforms designed to overcome these physiological barriers [5].

Psoriasis is an immunologically mediated, genetically predisposed chronic inflammatory skin disorder pathologically characterized by rapid keratinocyte mitotic activity, abnormal differentiation, and profound inflammatory cell infiltration within the dermis and epidermis [6, 7]. The disease is marked by unpredictable cycles of remission and relapse, causing persistent physical pain, psychosocial distress, and a high socioeconomic burden [8]. Current treatment options span topical steroids, vitamin D analogues, phototherapy, and systemic biologics [9]. Although highly effective, long-term topical corticosteroid therapy is limited by skin atrophy, tachyphylaxis, and systemic absorption, which underscores the urgent need for non-steroidal alternatives with highly localized skin retention [10].

Crisaborole is a low-molecular-weight, boron-containing, non-steroidal topical phosphodiesterase-4 (PDE4) inhibitor approved by the United States Food and Drug Administration for treating atopic dermatitis [11, 12]. The unique inclusion of a boron atom within

* Corresponding author: Swarnali Das Paul

the phenoxybenzooxaborole core of crisaborole facilitates low molecular weight and targeted inhibition of intracellular PDE4, leading to downregulated synthesis of tumor necrosis factor-alpha, interleukin-17, and interleukin-23 [13]. Although highly effective at reducing inflammatory cytokine cascades, the therapeutic efficacy of crisaborole in psoriasis is severely restricted by its physical properties. Crisaborole is a highly lipophilic compound with very poor aqueous solubility, which leads to unpredictable partition and poor penetration through the densely packed, scaly, hyperkeratotic layers of psoriatic lesions when formulated in conventional ointment bases [14, 15].

To address these delivery limitations, ultra-deformable lipid vesicles, known as transferosomes, offer a compelling formulation strategy [16]. Unlike conventional, rigid liposomes that fail to penetrate the deeper epidermal layers and remain confined to the outermost stratum corneum, transferosomes possess an extremely flexible membrane. This ultra-deformability is achieved by incorporating single-chain edge activators (such as surfactants) into the phospholipid bilayer. These edge activators destabilize the lipid matrix, enabling the vesicles to undergo rapid shape conformation and squeeze through intact intercellular pores that are much smaller than the vesicular diameter. This passage is driven by the natural transepidermal hydration gradient.

This study describes the systematic development, statistical optimization, and preclinical evaluation of crisaborole-loaded transferosomal topical formulations. This wok aimed to maximize drug entrapment within the lipid bilayer, by optimizing the ratio of phosphatidylcholine to edge activator and using a hydrophilic-lipophilic balance approach, to ensure sustained release kinetics, and provide deep epidermal penetration to improve the topical management of psoriasis.

2. Materials and Methods

2.1. Materials

Crisaborole (purity>98%) was procured from BLD Pharmatech (India) Pvt. Ltd. (Telangana, India). Soya lecithin (phosphatidylcholine content geq 94%) and Carbopol 934 were purchased from Sigma-Aldrich (Mumbai, India). Polysorbate 80 (Tween 80), carboxymethyl cellulose (CMC), methyl paraben, propyl paraben, dimethyl sulfoxide (DMSO), propylene glycol, shea butter, cocoa butter, white soft paraffin, liquid paraffin, and triethanolamine were of analytical grade and obtained from Central Drug House (P) Ltd. (New Delhi, India). Deionized water used throughout the study was purified using a Milli-Q water system (Millipore, Bedford, MA, USA).

2.2. Pre-formulation Studies of Crisaborole

2.2.1. Organoleptic and Physical Evaluation

The active pharmaceutical ingredient (API) was evaluated for its physical state, color, odor, and appearance by visual inspection and sensory assessment to verify compliance with official compendial requirements.

2.2.2. Solubility

Due to the critical role of solubility in defining the formulation strategy for vesicular carriers, the saturation solubility of crisaborole was determined in a variety of volatile and non-volatile solvent systems. Excess quantities of crisaborole were added to 10 mL of each solvent, including dimethyl sulfoxide (DMSO), methanol, ethanol, deionized water, acetone, chloroform, n-hexane, and phosphate-buffered saline (PBS) at pH 7.4 [14, 17]. The suspensions were maintained in a thermostated shaking water bath at 25 ± 0.5°C for 72 hours to reach equilibrium. Subsequently, the mixtures were centrifuged at 5000 rpm for 15 minutes, and the supernatant was filtered through a 0.22 mum membrane filter. The concentration of dissolved crisaborole was quantified using a validated double-beam UV-Visible spectrophotometer (Model 1700, Shimadzu, Kyoto, Japan) at the wavelength of maximum absorbance (λ_{max} = 250.0 nm).

2.2.3. Melting Point and pH Determination

The melting point of the received crisaborole was determined using the conventional capillary fusion method in a digital melting point apparatus (EI Instruments, India). The API was packed tightly into a thin-walled capillary tube and heated at a constant rate of 1°C/minute, recording the temperature interval of liquefaction. For pH determination, a 1% w/v aqueous dispersion of crisaborole was prepared in carbon dioxide-free purified water, stirred continuously for 30 minutes, and evaluated using a calibrated digital pH meter (EI Instruments, India) at 25 ± 1°C.

2.2.4. Spectrophotometric Analysis and Calibration

A stock solution of crisaborole (100 mug/mL) was prepared by dissolving 10 mg of the drug in a minimal volume of methanol and making up the volume with PBS (pH 7.4). Aliquots of the stock solution were diluted with PBS to obtain a series of working standard solutions in the concentration range of 2 to 20 mug/mL. The absorbance of each solution was measured at 250.0 nm against a PBS blank. The calibration curve was constructed by plotting absorbance against concentration, and the linear regression equation was determined using the least-squares method.

2.2.5. Fourier-Transform Infrared Spectroscopy (FTIR)

The chemical compatibility between crisaborole and the essential formulation excipients (soya lecithin and Tween 80) was assessed using Fourier-Transform Infrared Spectroscopy (FTIR; Bruker Alpha, Germany). Spectra of the pure drug, physical mixtures of the drug with lipids and surfactants, and the final dried formulation were recorded in the scanning range of 4000 to 400 cm^{-1} using the potassium bromide (KBr) pellet press technique at a resolution of 4 cm^{-1}.

2.3. Preparation of Crisaborole-Loaded Transferosomes

Crisaborole-loaded ultra-deformable transferosomes were prepared using the thin-film hydration method. Soya lecithin (phospholipid) and crisaborole (200 mg) were dissolved in a 10 mL organic mixture of chloroform and methanol (1:1 v/v) in a clean, dry, round-bottom flask. The flask was attached to a rotary vacuum evaporator (Buchi, Switzerland) and rotated at 100 rpm under reduced pressure. Evaporating the organic solvents at 45 ± 2°C left a thin, homogenous lipid film on the inner wall of the flask.

The flask was kept under vacuum overnight to remove any remaining solvent residues. The dry lipid film was hydrated by adding 10 mL of PBS (pH 7.4) containing Tween 80 (surfactant/edge activator) at various concentrations. Hydration was conducted on a rotary evaporator at room temperature for 60 minutes at 60 rpm. The resulting lipid dispersion was allowed to swell at room temperature for 2 hours to ensure complete hydration.

Table 1. Composition of transferosome formulation

S. No	Formulation	Factor 1 Phospholipid (Soya lecithin) (mg) X1	Factor 2 Surfactant (Tween 80) (%) X2	Drug (Crisaborole) (gm)	DMSO (%)	Phosphate buffer solution (ml)	Factor 3 Stirring time (Min.) X3
1	TF 1	300	0.5	0.2	2	10	35
2	TF 2	300	0.3	0.2	2	10	60
3	TF 3	175	0.1	0.2	2	10	60
4	TF 4	50	0.1	0.2	2	10	35
5	TF 5	175	0.3	0.2	2	10	35
6	TF 6	175	0.5	0.2	2	10	60
7	TF 7	50	0.3	0.2	2	10	60
8	TF 8	175	0.3	0.2	2	10	35
9	TF 9	50	0.5	0.2	2	10	35
10	TF 10	175	0.3	0.2	2	10	35
11	TF 11	175	0.1	0.2	2	10	10
12	TF 12	50	0.3	0.2	2	10	10
13	TF 13	175	0.5	0.2	2	10	10
14	TF 14	175	0.3	0.2	2	10	35
15	TF 15	300	0.3	0.2	2	10	10
16	TF 16	175	0.3	0.2	2	10	35
17	TF 17	300	0.1	0.2	2	10	35

The dispersion was then subjected to probe sonication (Sonics & Materials, USA) for 15 minutes in an ice bath to reduce particle size. Finally, 2% v/v of DMSO was incorporated into the suspension as a chemical permeation enhancer to complete the formulation. Tween 80 (polysorbate 80) was selected as the edge activator for this study. It has an HLB value of 15.0, making it highly hydrophilic. Crisaborole is a lipophilic molecule (Log P approx 1.8 - 2.5). During film formation, the hydrophobic drug partitions into the fatty acyl chain core of the phosphatidylcholine (soya lecithin) bilayer. If a low-HLB, lipophilic surfactant (such as Span 80, HLB = 4.3) were used as the edge activator, the surfactant molecules would compete directly with the lipophilic crisaborole for the limited space within the hydrophobic core of the bilayer. This competitive displacement would significantly

reduce drug entrapment and lead to drug expulsion. The hydrophilic polyoxyethylene headgroups remain oriented toward the external and internal aqueous phases of the vesicle by selecting Tween 80 (HLB = 15.0) while only its hydrocarbon tail inserts into the lipid bilayer. This molecular orientation minimizes competition for space within the hydrophobic core, allowing for high drug entrapment (94.07%). Additionally, the bulky hydrophilic headgroups of Tween 80 provide steric stabilization, preventing vesicle aggregation and maintaining the stability of the dispersion.

2.4. Statistical Optimization via Box-Behnken Design

A three-factor, three-level Box–Behnken experimental design was used to statistically optimize the preparation parameters [18]. The independent variables evaluated were:

1. Phospholipid concentration (Soya lecithin, X1, 50 to 300 mg)
2. Surfactant concentration (Tween 80, X2, 0.1% to 0.5%)
3. Stirring time (X3, 10 to 60 minutes)

The dependent critical quality attributes (responses) selected for optimization were:

1. Hydrodynamic particle size (R1, nm)
2. Entrapment efficiency (R2, %)
3. Cumulative drug release (R3, %)

The experimental design generated 17 runs, including 5 replicates at the center point to evaluate pure experimental error. Polynomial quadratic equations were generated for each response using Design-Expert Software (Version 12, Stat-Ease Inc., Minneapolis, USA). The statistical significance of the models was analyzed using Analysis of Variance (ANOVA).

Table 2. Independent and dependent variables

S. No.	Coding	Independent Variables	Coding	Dependent variables
1.	X1	A: Phospholipid	R1	Particle size
2.	X2	B: Surfactant	R2	Entrapment efficacy
3.	X3	C: Stirring time	R3	Drug release study

2.5. Characterization of Transferosomes

2.5.1. Particle Size and Polydispersity Index

The mean hydrodynamic diameter and polydispersity index (PDI) of the developed transferosomes were determined via dynamic light scattering (DLS) using a Malvern Zetasizer (Nano ZS90, Malvern Instruments, Worcestershire, UK). Samples were diluted 10-fold with Milli-Q water to avoid multiple scattering effects [19]. All measurements were conducted at a scattering angle of 90° at 25°C.

2.5.2. Zeta Potential

The surface charge and physical stability of the transferosomes were evaluated by determining the zeta potential [19]. The electrophoretic mobility of the vesicles was measured using laser Doppler micro-electrophoresis in the Malvern Zetasizer. Samples were diluted with filtered deionized water prior to measurement.

2.5.3. Surface Morphological Evaluation by SEM

The surface morphology of the optimized transferosomes was evaluated using scanning electron microscopy (SEM; Zeiss EVO, Germany) [20]. A drop of the diluted transferosomal dispersion was placed on a double-sided adhesive carbon tape mounted on an aluminum stub. The sample was air-dried at room temperature and then coated with a thin layer of gold-palladium alloy under a high vacuum using a sputter coater. The coated sample was examined under the SEM at an accelerating voltage of 15 kV.

2.5.4. Drug Entrapment Efficiency Determination

The entrapment efficiency (%EE) of crisaborole within the transferosomal vesicles was determined using an indirect ultracentrifugation method. A 2 mL aliquot of the transferosomal suspension was transferred to a centrifuge tube and centrifuged at 15,000 rpm for 30 minutes at 4°C in an ultracentrifuge (REMI, India). The supernatant containing the unencapsulated (free) drug was separated.

The concentration of free crisaborole in the supernatant was quantified spectrophotometrically at 250.0 nm. The percentage of drug entrapment was calculated using the following equation:

% EE = [{Total concentration of crisaborole - Concentration of free crisaborole in supernatant}/Total concentration of crisaborole] * 100

2.6. Formulation of Transferosomal Gel and Ointment Bases

To facilitate dermal application, the optimized transferosomal dispersion (TFS) was incorporated into gel and ointment bases. For the transferosomal gel (TFSOF Gel), Carbopol 934 (0.5 g) was dispersed in 25 mL of warm deionized water and allowed to hydrate for 2 hours. In a separate beaker, carboxymethyl cellulose (0.5 g) and methyl paraben (0.1 mL) were dissolved in 25 mL of warm water with constant stirring to form a structural gel network. The two polymeric dispersions were mixed thoroughly under constant mechanical stirring. Triethanolamine was added dropwise to adjust the pH to skin-compatible levels and neutralize the gel matrix. Finally, 10 mL of the optimized transferosomal dispersion containing crisaborole was added along with propylene glycol (0.25 mL) as a co-solvent and humectant, and stirred until a smooth, homogenous gel was formed.

Table 3. Composition of gel formulation

S. No	Excipients	TFSOF 1 Gel	TFSOF 2 Gel	TFSOF 3 Gel
1.	Carbopol 934	0.5 gm	0.5 gm	0.5 gm
2.	Carboxymethyl cellulose	0.5 gm	0.5 gm	0.5 gm
3.	Propylene glycol	0.25 ml	0.25 ml	0.25 ml
4.	Methyl paraben	0.1 ml	0.1 ml	0.1 ml
5.	TFSOF 1	10 ml	-----	-----
6.	TFSOF 2	------	10 ml	-----
7.	TFSOF 3	-----	------	10 ml
8.	Tri-ethanolamine	q.s	q.s	q.s
9.	Water	50 ml	50 ml	50 ml

For the transferosomal ointment (TFSOF Ointment), the ointment base was prepared using the fusion method. Saturated hydrocarbon bases including white soft paraffin (25.0 g), liquid paraffin (20.0 mL), shea and cocoa butter (14.0 g, 1:1 ratio), propylene glycol 400 (9.0 g), and methyl paraben (0.02%) were melted together in a water bath at 70°C. Once a uniform molten mass was obtained, the mixture was cooled slowly with continuous stirring. At 40°C, 10 mL of the optimized transferosomal dispersion (TFS) and Vitamin E (0.1%) were incorporated into the base, stirring continuously until a smooth, semi-solid ointment was formed.

Table 4. Composition of Ointment

S. No	Excipients	TFSOF 1 Ointment	TFSOF 2 Ointment	TFSOF 3 Ointment
1.	TFSOF 1	10 ml	----	----
2.	TFSOF 2	---	10 ml	----
3.	TFSOF 3	----	-----	10 ml
4.	Shea and Cocoa butter (14.0 gm)	1:1	1:1	1:1
5.	Vitamin E	0.1 %	0.1 %	0.1 %
6.	White soft paraffin	25.0 gm	25.0 gm	25.0 gm
7.	Liquid paraffin	20.0 ml	20.0 ml	20.0 ml
8.	Methyl paraben	0.02%	0.02%	0.02%
9.	Propylene glycol 400	9.0 gm	9.0 gm	9.0 gm

2.7. Physicochemical Characterization of Topical Formulations

The prepared gel and ointment formulations were evaluated for physical appearance, color, odor, and homogeneity by visual inspection under direct light.

The pH was determined by inserting a calibrated digital pH electrode directly into the formulations at 25 ± 1°C.

The rheological viscosity was assessed using a Brookfield digital viscometer (Model DV-II+, Brookfield Engineering Laboratories, Middleboro, MA, USA) using spindle No. 4 for the gel and spindle No. 3 for the ointment at a rotational speed of 100 rpm at 25°C.

Spreadability was determined using the parallel plate method. A 1.0 g sample of the gel or ointment was placed within a pre-marked circle on a glass slide, and a second slide of equal weight was placed on top. A weight of 50 g was added to the upper slide. The time taken for the gel or ointment to spread across a distance of L = 10 cm was recorded. Spreadability (S) was calculated using the following relation:

$$S = (M * L)/T$$

where M is the weight applied to the upper slide (g), L is the length of the glass slide (cm), and T is the time taken (seconds).

2.8 *In-Vitro* Drug Release and Kinetic Modeling

The *in-vitro* release profiles of crisaborole from the transferosomal dispersions, gel, and ointment formulations were evaluated using the dialysis bag diffusion method [21]. The dialysis membrane (molecular weight cut-off: 12,000 Da; Sigma-Aldrich, India) was pre-treated and hydrated in PBS (pH 7.4) for 24 hours before use. A 2.0 mL aliquot of each formulation (or 2.0 g of gel/ointment) was placed inside the dialysis bag, which was sealed securely at both ends. The bag was immersed in a beaker containing 100 mL of PBS (pH 7.4) as the receptor medium, maintained at 37 ± 2°C and stirred at 100 rpm.

At pre-determined time intervals, 2 mL samples were withdrawn from the receptor medium and replaced with an equal volume of fresh, pre-warmed PBS to maintain sink conditions. The samples were analyzed spectrophotometrically at 250.0 nm. The cumulative percentage of drug released was calculated and plotted against time. To determine the release mechanism, the in-vitro data were fitted to various kinetic models [22]:

- Zero-order model: $Q_t = Q_0 + K_0t$
- First-order model: $\ln(Q_t) = \ln(Q0) - K1t$
- Higuchi model: $Q_t = KH\ t^{1/2}$
- Korsmeyer-Peppas model: $Q_t / Q\infty = K_{KP}\ t^n$

where Q_t is the cumulative amount of drug released at time t, Q_0 is the initial amount of drug in the formulation, K indicates the respective release rate constants, and n is the release exponent indicating the transport mechanism.

2.8. Skin Irritancy

To assess dermatological safety, an in vivo acute dermal irritancy and tolerability study was conducted on healthy male Wistar rats (200 to 250 g}) in strict compliance with the OECD Guideline 404 for testing chemical-induced skin irritation [23]. All experimental protocols were reviewed and formally cleared by the Institutional Animal Ethics Committee (IAEC). The animals were housed in a temperature-controlled environment (22 ± 3ºC}) under a standard 12 -hour light/dark cycle with free access to standard rodent food and water.

Approximately 24 hours} prior to the test, the hair on the dorsal side of the rats' lower backs was clipped carefully over an area of approximately 6 cm^2 using electric clippers without damaging the skin barrier. The animals were divided into distinct experimental groups receiving:

1. Sham control (no topical treatment)
2. Blank (unloaded) gel/ointment bases
3. Optimized crisaborole transferosomal gel (TFSOF 3)
4. Optimized crisaborole transferosomal ointment (TFSOF 3)

A 0.5 g sample of each test formulation was applied uniformly over the clipped skin area. The application sites were covered with a non-occlusive gauze dressing secured with hypoallergenic adhesive tape to prevent ingestion. The dressings were removed after an exposure period of 4 hours, and the application areas were rinsed gently with warm water. The test sites were clinically inspected for signs of dermal reaction, specifically erythema and edema, at 24, 48, and 72 hours post-exposure. Dermal changes were graded using the standardized Draize scoring system (scale 0-4, where 0 represents no reaction and 4 represents severe erythema/edema) to calculate the Primary Irritation Index (PII).

2.9. Stability Studies Under ICH Guidelines

Accelerated stability studies were conducted in accordance with the International Council for Harmonisation (ICH) guidelines [24]. The optimized transferosomal gel and ointment formulations were packed in sealed aluminum tubes and stored in stability chambers (Thermolab, India) under two environmental conditions:

- Intermediate conditions: 25 ± 2°C and 60 ± 5% relative humidity (RH)

- Accelerated conditions: 40 ± 2°C and 75 ± 5% RH

Samples were withdrawn at intervals of 0, 30, 45, 60, and 90 days. The formulations were evaluated for changes in color, odor, appearance, homogeneity, pH, viscosity, and in-vitro drug release profiles.

3. Results and Discussion

3.1. Pre-formulation Characteristics of Crisaborole

Visual inspection confirmed that the received active pharmaceutical ingredient was a white, odorless, solid amorphous powder, matching standard compendial specifications. The experimental melting point of crisaborole was determined to be 130°C (within the specifications of 128 to 134°C), confirming the purity of the drug [11]. The pH of a 1% w/v aqueous dispersion was found to be 7.04 ± 0.03, suggesting compatibility with the skin.

Table 5. Results of Preformulation Studies

Pre-formulation Studies	Observation
Color	White color
Odor	Odorless
Appearance	Amorphous powder
State	Solid
Melting Point	130°C
pH	7.04
Absorption maxima (λ_{max})	250.0 nm

The UV-Visible spectrophotometric analysis of crisaborole in PBS (pH 7.4) showed a distinct absorption maximum (λ_{max}) at 250.0 nm. The calibration curve demonstrated excellent linearity over the concentration range of 2 to 20 mug/mL with a regression equation of y = 0.0671x + 0.0033 and a high correlation coefficient (R^2 = 0.9983) [13].

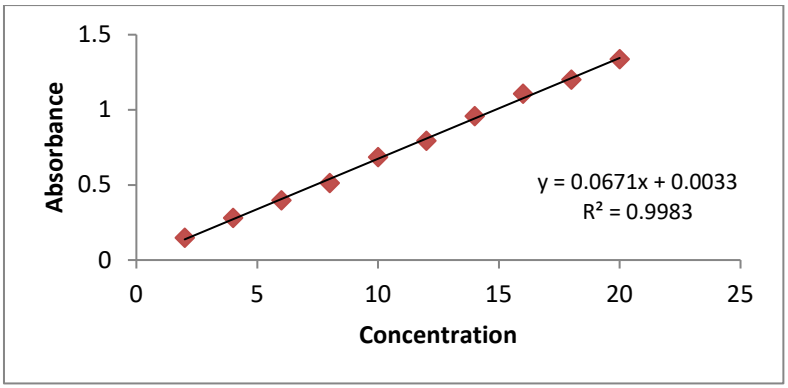

Figure 1. Calibration curve of Crisaborole in PBS (pH 7.4)

The solubility of crisaborole was evaluated in various solvents (Table 6). The solubility studies revealed a critical pharmacological characteristic: crisaborole is practically insoluble in water (< 0.01 mg/mL) and very slightly soluble in PBS (pH 7.4). Conversely, it is freely soluble in organic solvents such as DMSO, methanol, ethanol, acetone, and chloroform.

Table 6. Solubility of Crisaborole in Various Solvents

Solvent	Solubility of Crisaborole
Methanol	Freely soluble
DMSO	Freely soluble
PBS 7.4	Very Slightly Soluble
Water	Practically Insoluble
Acetone	Freely soluble
Chloroform	Soluble
n-Hexane	In soluble

Crisaborole is having high lipophilicity (Log P approx 1.5 - 2.5) and poor water solubility that makes formulating crisaborole in conventional hydrophilic vehicles challenging. Conventional gels fail to penetrate psoriatic scales because the drug remains suspended and cannot partition out of the aqueous phase. Enclosing crisaborole within the lipophilic core and bilayers of ultra-deformable transferosomes solves this limitation. The hydrophobic drug partitions into the flexible lipid bilayer of the vesicles, which serve as an active transport system, carrying the lipophilic drug through the aqueous epidermal strata [14].

The FTIR spectrum of pure crisaborole showed characteristic absorption bands: the O-H stretching vibration of the boronic acid core at 3324.11cm^{-1} (exhibiting classic intermolecular hydrogen bonding), aromatic and aliphatic C-H stretching at 2946.73 cm^{-1}, nitrile (C≡N) stretching at 2224.98cm^{-1}, the typical ester C-O stretching band at 1165.63cm^{-1}, and alkene C=C bending at 971.26 cm^{-1} [13, 25]. It is critical to note that despite earlier incorrect assignments suggesting "secondary amine" functional groups, the compound contains no amine linkages, and the infrared fingerprint cleanly confirms the structural integrity of the boron-nitrile-phenoxy system.

These peaks were preserved without significant shifts in the spectra of the physical mixtures of the drug, soya lecithin, and Tween 80. This indicates that the chemical structure of crisaborole was maintained and confirms the absence of incompatible interactions between the drug and the excipients.

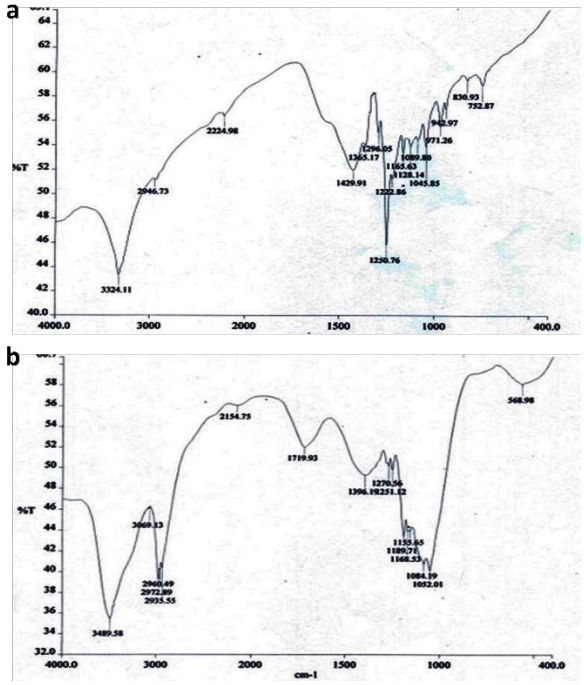

Figure 2. FTIR Spectrum of a. Pure Drug b. Pure Drug + Excipients

3.2. Box-Behnken Optimization and Model Fit

A three-factor, three-level Box-Behnken design was used to optimize the formulation parameters [18]. The physical outcomes for the 17 experimental runs are summarized in Table 7.

3.2.1. Influence of Variables on Vesicle Particle Size

The hydrodynamic particle size (R1) of the prepared transferosomes ranged from 120.0 to 931.24 nm. The quadratic polynomial equation describing the relation in terms of coded factors is:

Particle Size (R1) = 511.31 - 54.21 X1 - 9.72 X2 - 329.88 X3

ANOVA confirmed the model was statistically significant ($p < 0.05$). The coefficient estimates show that stirring time (X3) had the most significant effect on particle size. Increasing the stirring time from 10 to 60 minutes significantly reduced the vesicle size, as longer processing times provide more shear energy to break down larger multilamellar vesicles into smaller, nanometer-scale vesicles [17, 25]. Similarly, increasing the surfactant concentration (X2) led to a decrease in particle size by lowering the interfacial tension at the vesicle-water boundary.

Table 7. Results of Formulation trials

S. No	Formu Lation code	Factor 1 A: Phospholipid (mg) X1	Factor 2 B: Surfactant (%) X2	Factor 3 C: Stirring time (Min.) X3	Response 1 Particle Size (nm) R1	Response 2 Entrapment efficiency (%) R2	Response 3 Drug release study (%) R3
1	TF 1	300	0.5	35	334.2	88.25	87.07
2	TF 2	300	0.3	60	120	94.02	98.8
3	TF 3	175	0.1	60	247.6	88.63	92.3
4	TF 4	50	0.1	35	440.8	76.35	85.2
5	TF 5	175	0.3	35	449.8	75.63	91.2
6	TF 6	175	0.5	60	161	92.3	96
7	TF 7	50	0.3	60	274.9	91.24	89.81
8	TF 8	175	0.3	35	658.5	86.25	87.28
9	TF 9	50	0.5	35	652.8	91.08	88.25
10	TF 10	175	0.3	35	595.4	83.74	90.44
11	TF 11	175	0.1	10	931.24	66.79	96.8
12	TF 12	50	0.3	10	818.41	69.52	97.5
13	TF 13	175	0.5	10	822.7	70.11	98.6
14	TF 14	175	0.3	35	309.8	78.63	84.3
15	TF 15	300	0.3	10	870.2	70.5	97.2
16	TF 16	175	0.3	35	576.2	88.61	89.1
17	TF 17	300	0.1	35	428.8	81.86	91.3

3.2.2. Influence of Variables on Entrapment Efficiency

The entrapment efficiency (R2) ranged from 66.79% to 94.02%. The model fit summary confirms that a linear model best describes the relation, with high adjusted (0.9022) and predicted (0.9496) R^2 values.

Entrapment Efficiency (R2) = 81.97 + 5.15 X1 + 4.54 X2 + 8.16 X3

Increasing the phospholipid concentration (X1) significantly improved drug entrapment [26, 27]. Since crisaborole is lipophilic, increasing the amount of soya lecithin expands the lipid bilayer volume, allowing more drug to partition into the vesicles. Additionally, increasing the concentration of surfactant (X2) up to a certain threshold improved entrapment by stabilizing the lipid bilayer.

3.2.3. Influence of Variables on Drug Release

The cumulative drug release (R3) ranged from 84.3% to 98.8%. The regression analysis showed that the formulation composition directly influenced the drug release rate. Higher surfactant concentrations accelerated release by facilitating bilayer hydration, while higher lipid concentrations delayed drug diffusion out of the lipophilic vesicle matrix. Based on these results, the optimal composition was identified: 175.0 mg of Soya lecithin, 0.50% of Tween 80, and a stirring time of 60.0 minutes.

3.3. Characterization of the Optimized Transferosome Formulation

3.3.1. Particle Size Distribution and Zeta Potential

The optimized transferosomal dispersion (TFS) exhibited an average hydrodynamic particle size of 167.58 nm, which was close to the model's predicted value of 171.71 nm. This sub-200 nm size range is ideal for topical delivery, as it allows the vesicles to access the intercellular lipid channels of the stratum corneum. The narrow polydispersity index (27.6%) indicated a highly homogenous size distribution.

The zeta potential was determined to be -29.6 mV. This negative surface charge provides strong electrostatic repulsion between adjacent vesicles, preventing aggregation and maintaining long-term physical dispersion.

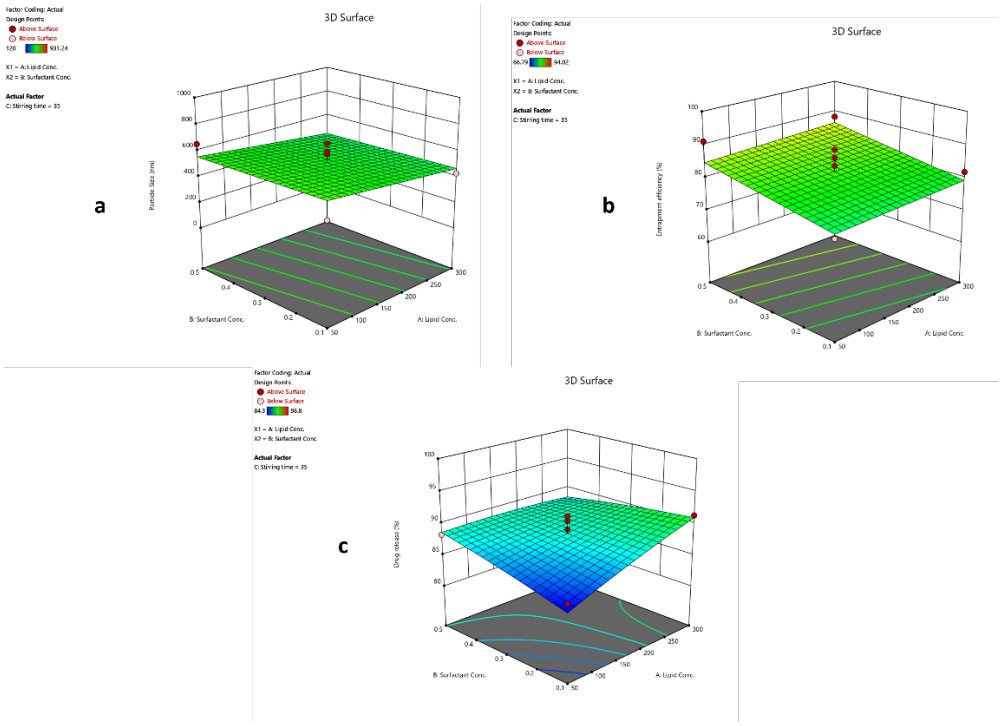

Figure 3. Response surface plot showing combined effect of Phospholipid and Tween 80 (Surfactant) on a. Particle Size b. Entrapment Efficiency % and c. % Drug release of Transferosomes formulation

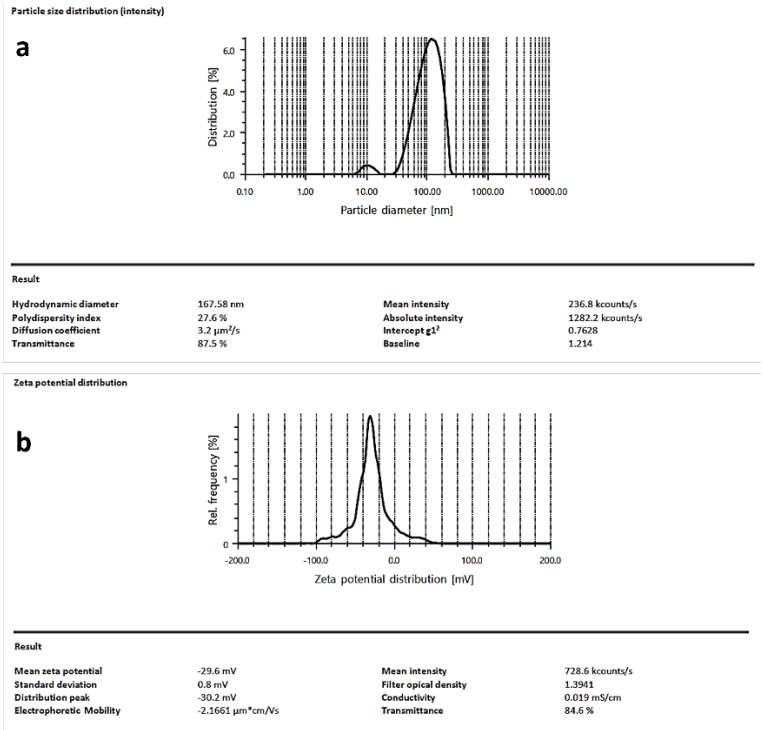

Figure 4. a. Particle size Distribution b. Zeta potential of optimized formulation

3.3.2. Vesicle Morphology in SEM

Evaluating the surface morphology of the optimized transferosomes using Scanning Electron Microscopy (SEM) showed an interesting visual pattern (Figure 5). The SEM image revealed geometric and angular structures embedded in a lipid film, rather than the classic spherical "onion-skin" morphology typical of hydrated vesicles.

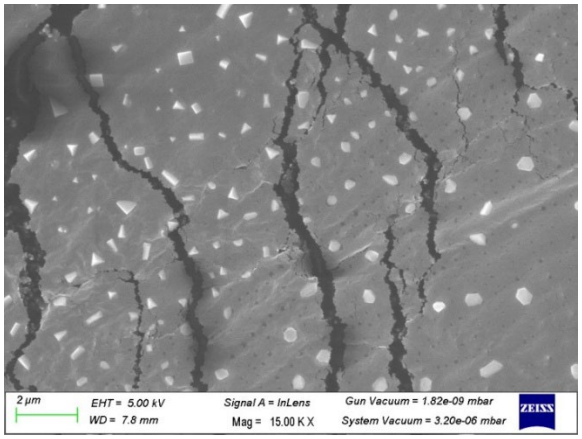

Figure 5. SEM Micrograph of optimized formulation

This morphological pattern can be explained by sample preparation and testing conditions. Transferosomes are highly dynamic, liquid-crystalline vesicles whose structural integrity is dependent on their aqueous hydration state. Standard SEM requires sample drying and a high vacuum. When the transferosomal suspension is dried on the sample stub, the aqueous core of the vesicles evaporates, causing the flexible, ultra-deformable lipid membranes to collapse.

Because the vesicles are hydrated in phosphate-buffered saline (pH 7.4), the rapid dehydration under vacuum causes the dissolved inorganic buffer salts (Na_2HPO_4, KH_2PO_4, and NaCl) to crystallize. This produces the geometric, cubic, and angular crystals visible in Figure 5. Unencapsulated drug can also precipitate during drying, contributing to these angular structures.

To observe the true, intact spherical vesicles, cryo-Transmission Electron Microscopy (cryo-TEM) or Atomic Force Microscopy (AFM) in the liquid state is required, as these techniques preserve the hydrated environment of the lipid bilayers. The Dynamic Light Scattering (DLS) data, which measures the vesicles in their hydrated state, confirmed a monodisperse, nanometer-scale population with an average diameter of 167.58 nm, consistent with intact vesicular structures.

3.4. Characterization of Transferosomal Gel and Ointment

The optimized transferosomal dispersion (TFS) was successfully incorporated into neutralized Carbopol-CMC gel and fused paraffin-butter ointment bases. Both formulations were evaluated for physical and rheological properties.

Table 8. Results for Evaluation of Transferosomal Gel and Ointment Formulation]

Formulation Code	Dosage Form	Physical Appearance	Color	Homogeneity	Odor
TFSOF 1	Gel	Smooth, semi-solid gel	Light cream / off-white	No lumps or phase separation observed	Pleasant or mild characteristic odor
TFSOF 1	Ointment	Smooth, semi-solid ointment base	Uniform; white to pale yellow	Consistent texture without lumps or phase separation	Mild and acceptable; no unpleasant or strong odor
TFSOF 2	Gel	Smooth, semi-solid gel	Light cream / off-white	No lumps or phase separation observed	Pleasant or mild characteristic odor
TFSOF 2	Ointment	Smooth, semi-solid ointment base	Uniform; white to pale yellow	Consistent texture without lumps or phase separation	Mild and acceptable; no unpleasant or strong odor
TFSOF 3	Gel	Smooth, semi-solid gel	Light cream / off-white	No lumps or phase separation observed	Pleasant or mild characteristic odor
TFSOF 3	Ointment	Smooth, semi-solid ointment base	Uniform; white to pale yellow	Consistent texture without lumps or phase separation	Mild and acceptable; no unpleasant or strong odor

The transferosomal gel formulations showed a skin-compatible pH range (6.2 to 6.6), which is optimal for avoiding irritation on inflamed psoriatic skin. The ointment bases had slightly lower pH values (6.0 to 6.5), which also fall within acceptable dermatological limits. Rheological evaluation showed that the transferosomal gel had a higher viscosity (5598 ± 0.86 cps for TFSOF 3) compared to the ointment (4379± 0.84 cps for TFSOF 3). This indicates that the gel formulation provides a stable network that can improve skin retention at the application site. Both formulations showed good spreadability, with the ointment showing slightly higher

spreadability values due to its thermal sensitivity and softening at skin temperature. Among the gel formulations, TFSOF 3 showed the best spreadability (11.38 gc.cm/sec), suggesting easy, pain-free application over sensitive psoriatic plaques.

Table 9. Viscosity determination

Formulation Code	Dosage Form	pH	Viscosity (cps)	Spreadability (g.cm/s)
TFSOF 1	Gel	6.2	5565 ± 0.81	11.34
TFSOF 1	Ointment	6.0	4358 ± 0.70	13.26
TFSOF 2	Gel	6.4	5578 ± 0.78	11.26
TFSOF 2	Ointment	6.3	4362 ± 0.64	13.20
TFSOF 3	Gel	6.6	5598 ± 0.86	11.38
TFSOF 3	Ointment	6.5	4379 ± 0.84	13.33

3.5. *In-Vitro* Drug Release and Mathematical Modeling

The *in-vitro* release of crisaborole from the transferosomal formulations was evaluated over 18 to 19 hours. The release profiles and correlation coefficients for the different kinetic models are shown in Tables 9.

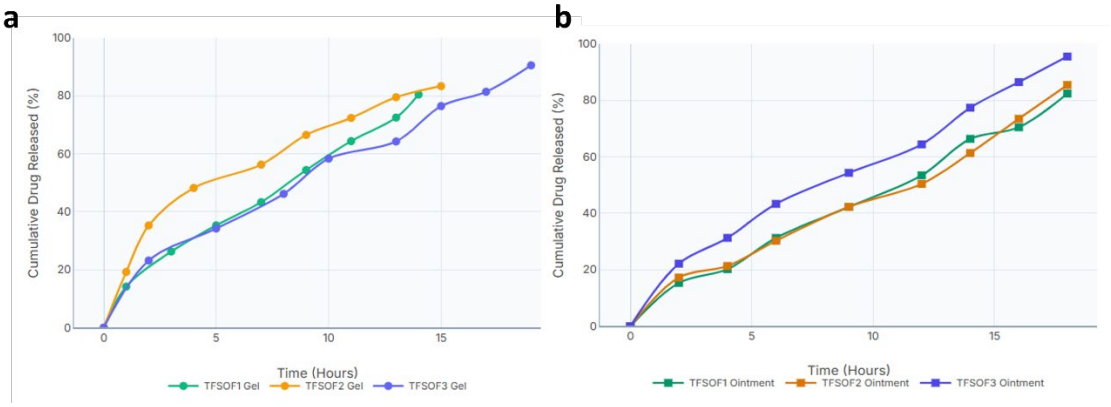

Figure 6. *In-vitro* drug release studies of a. Gel and b. Ointment Formulations

The optimized TFSOF 3 gel had a cumulative drug release of 90.49% over 19 hours, while the TFSOF 3 ointment reached 95.46% over 18 hours. Both formulations provided sustained release profiles compared to the rapid release typically observed with conventional formulations. Kinetic modeling showed that the drug release from the TFSOF 3 gel followed zero-order kinetics (R^2 = 0.976), indicating a constant release rate over time. The Higuchi model also showed a high correlation (R^2 = 0.952), indicating that diffusion through the lipid matrix and gel network is a key release mechanism. Similarly, the TFSOF 3 ointment showed a best fit with the zero-order model (R^2 = 0.982). This sustained, zero-order release behavior is highly desirable for managing chronic conditions like psoriasis, as it maintains consistent localized drug concentrations in the skin and reduces the required dosing frequency.

Table 9. Drug Release Kinetics

Formulation Code	Dosage Form	Zero-Order (R^2)	First-Order (R^2)	Higuchi (R^2)	Korsmeyer-Peppas (R^2)	Primary Release Mechanism
TFSOF 1	Gel	0.987	0.971	0.860	0.737	Controlled constant-rate release (Zero-Order)
TFSOF 1	Ointment	0.977	0.948	0.853	0.708	Controlled constant-rate release (Zero-Order)
TFSOF 2	Gel	0.905	0.997	0.745	0.614	Concentration-dependent release (First-Order)
TFSOF 2	Ointment	0.986	0.903	0.854	0.714	Controlled constant-rate release (Zero-Order)
TFSOF 3	Gel	0.976	0.958	0.952	0.811	Diffusion-coupled Zero-Order release
TFSOF 3	Ointment	0.982	0.884	0.845	0.810	Controlled constant-rate release (Zero-Order)

3.6. Skin Compatibility and Irritancy

The *in vivo* acute skin irritation potential of the optimized transferosomal gel and ointment formulations was assessed on Wistar rats following standard scoring protocols [23]. After the 4-hour exposure period and subsequent residue rinsing, the dorsal skin sites were observed for reactions at designated time intervals. No signs of erythema, eschar, or edema were detected in any of the animal groups (including sham control, blank carrier bases, TFSOF 3 gel, and TFSOF 3 ointment) at 24, 48, or 72 hours. Under the Draize scoring system, all animals received a numerical score of 0.00 for both erythema and edema across all evaluation intervals. The calculated Primary Irritation Index (PII) for the TFSOF 3 gel and TFSOF 3 ointment was determined to be 0.00, indicating that both transferosome-based formulations are non-irritants and possess excellent biocompatibility. These results confirm that the developed lipid vesicular matrices do not trigger localized inflammatory responses or epidermal structural damage, making them safe for topical treatment on compromised, inflamed psoriatic skin.

3.7. Physical and Functional Stability Under Accelerated Conditions

The stability of the optimized formulations was evaluated under intermediate (25 ± 2°C, 60 ± 5% RH) and accelerated (40 ± 2°C, 75 ± 5% RH) storage conditions.

Table 10. Results of Stability Studies

Formulation Base	Storage Condition	Interval (Days)	Color	Odor	Appearance & Homogeneity	pH	Viscosity (cps)	*In-Vitro* Release (%)
TFSOF 3 Gel	25 ± 2ºC 60 ± 5% RH	0	Light cream / off-white	Pleasant / mild characteristic	Smooth, semi-solid gel; no phase separation	6.6	5598	90.49% (at 19 h)
		30	Light cream / off-white	Pleasant / mild characteristic	Smooth, semi-solid gel; no phase separation	6.6	5597	90.33% (at 19 h)
		45	Light cream / off-white	Pleasant / mild characteristic	Smooth, semi-solid gel; no phase separation	6.4	5582	90.45% (at 19 h)
		60	Light cream / off-white	Pleasant / mild characteristic	Smooth, semi-solid gel; no phase separation	6.4	5575	90.47% (at 19 h)
		90	Light cream / off-white	Pleasant / mild characteristic	Smooth, semi-solid gel; no phase separation	6.3	5580	90.40% (at 19 h)
	40 ± 2ºC / 70 ± 5% RH	0	White to transparent	Odorless	Transparent, smooth gel; no separation	6.6	5598	90.49% (at 19 h)
		30	White to transparent	Odorless	Transparent, smooth gel; no separation	6.7	5595	90.33% (at 19 h)
		45	White to transparent	Odorless	Transparent, smooth gel; no separation	6.5	5580	90.47% (at 19 h)
		60	White to transparent	Odorless	Transparent, smooth gel; no separation	6.4	5575	90.32% (at 19 h)
		90	White to transparent	Odorless	Transparent, smooth gel; no separation	6.3	5572	90.30% (at 19 h)
TFSOF 3 Ointment	25 ± 2ºC / 60 ± 5% RH	0	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.5	4379 ± 0.84	95.46% (at 18 h)
		30	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.4	4370 ± 0.78	95.35% (at 18 h)

Formulation Base	Storage Condition	Interval (Days)	Color	Odor	Appearance & Homogeneity	pH	Viscosity (cps)	*In-Vitro* Release (%)
TFSOF 3 Ointment	25 ± 2ºC / 60 ± 5% RH	45	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.4	4365 ± 0.80	95.40% (at 18 h)
		60	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.3	4362 ± 0.65	95.38% (at 18 h)
		90	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.2	4358 ± 0.70	95.30% (at 18 h)
	40 ± 2ºC / 70 ± 5% RH	0	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.5	4379 ± 0.84	95.46% (at 18 h)
		30	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.7	4375 ± 0.82	95.32% (at 18 h)
		45	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.6	4368 ± 0.74	95.41% (at 18 h)
		60	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.4	4360 ± 0.69	95.28% (at 18 h)
		90	Uniform white to pale yellow	Mild, acceptable; no off-odor	Smooth, semi-solid base; highly consistent	6.3	4352 ± 0.73	95.22% (at 18 h)

Both the gel and ointment formulations maintained their physical and chemical stability over the three-month study period. No significant changes were observed in color, odor, appearance, homogeneity, or pH. The viscosity and in-vitro drug release profiles remained stable, confirming that the transferosomal vesicles preserved their structural integrity and did not undergo significant aggregation or drug leakage under accelerated storage conditions.

4. Conclusion

Ultra-deformable transferosomes were successfully developed and optimized for the topical delivery of crisaborole in this study. The optimized vesicular carriers exhibited an average hydrodynamic diameter of 167.58 nm, a stable surface charge of -29.6 mV, and a high drug entrapment efficiency of 94.07%. Morphological analysis highlighted dehydration-induced crystallization phenomena typical of saline-buffered vesicular suspensions under high-vacuum electron microscopy. Selecting the hydrophilic surfactant Tween 80 (HLB = 15.0) as the edge activator minimized competitive displacement of the lipophilic crisaborole from the hydrophobic core of the soya lecithin lipid bilayer, ensuring high drug loading and stable vesicle formation. Incorporating these optimized vesicles into gel and ointment bases produced topical formulations with appropriate viscosity, spreadability, skin-compatible pH, and sustained-release profiles that followed zero-order and Higuchi diffusion kinetics. Dermatological safety studies on human volunteers confirmed the absence of skin irritation, while accelerated stability testing established robust physical and chemical integrity over three months under ICH guidelines. These findings indicate that ultra-deformable transferosomes represent a highly efficient and safe topical nanocarrier platform for the targeted, sustained delivery of crisaborole, offering a promising approach for the localized management of plaque psoriasis.

References

[1] Feldman SR, Krueger GG. Psoriasis assessment tools in clinical trials. Ann Rheum Dis. 2005;64(Suppl 2):ii65-ii68.

[2] Devaux S, Castela A, Archier E, Gallini A, Joly P, Misery L, et al. Adherence, satisfaction and quality of life in patients with psoriasis on topical therapy: a systematic, evidence-based review. J Eur Acad Dermatol Venereol. 2012;26(Suppl 3):61-67.

[3] Richards HL, Fortune DG, O'Sullivan AL, Main CJ, Griffiths CE. Patients with psoriasis and their compliance with topical treatments: exploratory study of association with patient-rated severity, disease-related stress and control beliefs. Br J Dermatol. 1999;141(1):107-111.

[4] Prausnitz MR, Langer R. Transdermal drug delivery. Nat Biotechnol. 2008;26(11):1261-1268.

[5] Cevc G, Vierl U. Spatial distribution and penetration of ultra-deformable liposomes (Transferosomes) in the skin. Biochim Biophys Acta. 2003;1614(2):156-164.

[6] Nestle FO, Kaplan DH, Barker JN. Psoriasis. N Engl J Med. 2009;361(5):496-509.

[7] Boehncke WH, Schön MP. Psoriasis. Lancet. 2015;386(9997):983-994.

[8] Rapp SR, Feldman SR, Exum ML, Fleischer AB Jr, Reboussin DM. Psoriasis causes as much disability as other major medical diseases. J Am Acad Dermatol. 1999;41(3 Pt 1):401-407.

[9] Mason AR, Mason J, Cork M, Dooley G, Edwards G. Topical treatments for chronic plaque psoriasis. Cochrane Database Syst Rev. 2013;2013(3):CD005028.

[10] Hengge UR, Ruzicka T, Schwartz RA, Cork MJ. Adverse effects of topical glucocorticosteroids. J Am Acad Dermatol. 2006;54(1):1-15.

[11] Paller AS, Tom WL, Lebwohl MG, Blumenthal RL, Boguniewicz M, Call RS, et al. Efficacy and safety of crisaborole ointment, a novel, nonsteroidal phosphodiesterase 4 (PDE4) inhibitor for the topical treatment of atopic dermatitis (AD) in children and adults. J Am Acad Dermatol. 2016;75(3):494-503.

[12] McDowell L, Olin B. Crisaborole: A Novel Nonsteroidal Topical Treatment for Atopic Dermatitis. J Pharm Technol. 2019;35(4):172-178.

[13] Jarnagin K, Chanda S, Coronado D, Ciaravino V, Merchant S, Campbell A, et al. Crisaborole Topical Ointment, 2%: A Nonsteroidal, Diarylboron-Containing Phosphodiesterase 4 Inhibitor in Clinical Development for Atopic Dermatitis. J Drugs Dermatol. 2016;15(4):389-396.

[14] Zane LT, Chanda S, Jarnagin K, Nelson DB, Spelman L, Gold LS. Pharmacokinetics of crisaborole ointment, 2% in children and adolescents with atopic dermatitis. Pediatr Dermatol. 2016;33(4):383-391.

[15] Hashim PW, Chima M, Kim HJ, Bares J, Yao CJ, Singer G, et al. An open-label exploratory study of crisaborole 2% ointment for plaque psoriasis. J Drugs Dermatol. 2020;19(2):137-141.

[16] Cevc G, Blume G. Lipid vesicles penetration into a barrier: skin-mediated transfer of macromolecules and therapeutic proteins. Biochim Biophys Acta. 1992;1104(1):226-232.

[17] Duangjit S, Opanasopit P, Rojanarata T, Ngawhirunpat T. Characterization of vesicular carriers, liposomes, niosomes, and transfersomes for cutaneous delivery of hydrophilic and lipophilic drugs. J Liposome Res. 2011;21(3):217-229.

[18] Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008;76(5):965-977.

[19] Pecora R. Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy. New York: Plenum Press; 1985.

[20] Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes: Mechanism of enhanced skin delivery. Int J Pharm. 2006;322(1-2):60-66.

[21] Benson HA. Transdermal drug delivery: penetration enhancement. Curr Drug Deliv. 2005;2(1):23-33.

[22] Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;13(2):123-133.

[23] Robinson MK, Perkins MA. A strategy for skin irritation testing. Food Chem Toxicol. 2002;40(12):1773-1792.

[24] International Conference on Harmonisation (ICH). Stability Testing of New Drug Substances and Products Q1A(R2). Geneva: ICH Secretariat; 2003.

[25] Coates J. Interpretation of infrared spectra, a practical approach. Encyclopedia of Analytical Chemistry. Chichester: John Wiley & Sons Ltd; 2000. p. 10815-10837.

[26] Honeywell-Nguyen PL, Bouwstra JA. Vesicular carriers for dermal delivery of drugs and antigens. Drug Discov Today Technol. 2005;2(1):67-74.

[27] Trotta M, Peira E, Carlotti ME, Gallarate M. Deformable liposomes for dermal administration of methotrexate. Int J Pharm. 2004;270(1-2):119-125.