IJISEM
INTERNATIONAL JOURNAL OF INNOVATIONS IN SCIENCE ENGINEERING AND MANAGEMENT

OPEN ACCESS

Volume: 5

Issue: 2

Month: April

Year: 2026

ISSN: 2583-7117

Published: 11.04.2026

Citation:

Prabhat Yadav, Dr. Brajesh Kumar Arjariya, Praveen Bhawsar "Development and Characterization of Crisaborole-Loaded Liposomal Drug Delivery System for Enhanced Topical and Antibacterial" International Journal of Innovations in Science Engineering and Management, vol. 5, no. 2, 2026, pp. 61-73

DOI:

10.69968/ijisem.2026v5i261-73

Development and Characterization of Crisaborole-Loaded Liposomal Drug Delivery System for Enhanced Topical and Antibacterial

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Abstract

The present study focuses on the preparation, development, and characterization of a liposphere formulation containing Crisaborole to enhance its solubility, dermal delivery, and antibacterial efficacy. The formulation was developed using the thin-film hydration method with soya lecithin. Pre-formulation studies confirmed suitable physicochemical properties, with a melting point of 131°C, pH 5.3, and λmax at 250 nm, showing good linearity (R^2 = 0.9951). The optimized formulation exhibited a particle size of 43.07 nm, zeta potential of -18.0 mV, and high entrapment efficiency (93.48%), indicating stability and uniformity. SEM analysis confirmed spherical morphology. In-vitro drug release followed zero-order and Higuchi kinetics, suggesting controlled diffusion. Stability studies demonstrated minimal changes under refrigerated conditions. The formulation showed enhanced antibacterial activity against Staphylococcus aureus and E. coli compared to the pure drug. Overall, the developed liposphere system proved to be a promising approach for improved topical delivery and therapeutic effectiveness of Crisaborole.

Keywords; Crisaborole, Liposphere formulation, Thin-film hydration, Entrapment efficiency, Antibacterial activity.

INTRODUCTION

Humans have relied on plants for medicinal purposes since ancient times, with herbal knowledge evolving over thousands of years. Phytoconstituents or bioactive compounds derived from plants have demonstrated significant therapeutic potential in treating diseases such as cancer, cardiovascular, and neurodegenerative disorders (Alexander et al., 2014). However, their clinical effectiveness is often limited due to poor physicochemical, pharmacokinetic, and pharmacodynamic properties, including low solubility, poor bioavailability, chemical instability, and inadequate permeability across biological membranes (Gupta et al., 2019).

Many phytoconstituents, such as flavonoids, tannins, terpenoids, and polyphenols, despite being water-soluble, exhibit poor absorption due to their large molecular size or inability to diffuse through lipid-rich intestinal membranes (Khogta et al., 2020). Others, like silybin, triterpenes, ginkgolides, bilobalide, and curcuminoids, suffer from low aqueous solubility, resulting in sub-therapeutic drug levels. These challenges highlight the need for formulations that maintain an optimal balance between hydrophilicity and lipophilicity for effective drug delivery (Gupta et al., 2019).

Recent advancements focus on the development of novel drug delivery systems (NDDS), including nano-sized particulate, colloidal, and vesicular carriers. These systems, typically ranging from 1 to 100 nm, integrate principles from biotechnology, polymer science, and pharmaceutical sciences to enhance the

delivery of bioactive compounds. Examples include liposomes, niosomes, phytosomes, nanoemulsions, microspheres, hydrogels, and self-emulsifying drug delivery systems (Mudie et al., 2010).

Encapsulation of herbal actives into such carriers improves solubility, stability, bioavailability, and targeted delivery while reducing metabolic degradation and toxicity. These systems also enhance patient compliance, particularly in pediatric and geriatric populations (Athanasakoglou and Kampranis 2019). Among these, liposomes and self-emulsifying systems are considered the most promising for herbal drug delivery. Overall, modern nanomedicine approaches offer significant potential to overcome the limitations of conventional herbal formulations and improve therapeutic outcomes (Gupta et al., 2019)

Novel strategies for delivery of herbal extract/ bioactive

- **Vesicular nano-carrier drug delivery system:** In the current scenario drug targeting and desired (sustained/controlled) delivery are key requirements to improve the quality of therapy. Researchers have focused on formulation studies of vesicular nanocarrier systems like Phytosomes, Liposomes, Niosomes, Transferosomes as these are promising materials to overcome the shortcomings associated with conventional medicines (Chaudhari and Randive 2020).
- **Colloidal particulate drug delivery system:** Nanotechnology deals with the size range of one thousand millionths of a particular unit i.e., 1 nm = 109 m, which offers an advanced drug delivery system for preventing and treating various diseases (Kumar et al., 2020). The different types of colloidal particulate drug delivery systems like Solid lipid nanoparticles, Metal nanoparticles, Microspheres and Emulsions.
- **Other drug delivery system:** Besides vesicular and colloidal systems hydrogels and cyclodextrin contributes as another drug delivery system (Ijaz et al., 2018; Pandeyet al., 2021).

Vesicular nano-carrier drug delivery system

Phytosome

Phytosome is a patented technology developed by INDENA which is a leading Italian manufacturing company of pharmaceuticals and nutraceuticals (Alexander et al., 2016). This technology has emerged as an advanced herbal drug delivery system with improved stability, bioavailability and target specificity of active plant constituents. According to the known definition, it consists of two words “Phyto” and “Some” here “Phyto” means plant and “Some” means cell-like structure (Katouzian et al., 2017).

Liposome

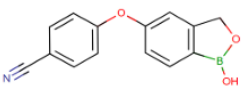
Liposomes are spherical shaped vesicular drug delivery systems comprising of concentric bilayers capable of encapsulating both hydrophilic and lipophilic material in a single structure (Alexander et al., 2016). They are self-assembled lipid bilayer membranes vesicles which are formed by phospholipids and cholesterol. This colloidal system is designed with a unique framework by interaction with water, polar lipids and form self-organized colloidal particles. As drug carriers, liposomes are extremely versatile. They can carry hydrophobic drugs within the non-polar lipid membrane core and hydrophilic drugs in the aqueous interior pocket consequently; the water-soluble compounds are entrapped in the aqueous compartment and lipid soluble compounds aggregate in the lipid section. The size of the liposomes ranges from 50 nm to more than 1 µm, depending upon their respective composition and preparation method (Sakai Kato et al., 2019).

DRUG PROFILE

Generic Name: Crisaborole

General Introduction: Crisaborole is a novel oxaborole approved by FDA on December 14, 2016 as Eucrisa, a topical treatment of for mild to moderate atopic dermatitis. This non-steroidal agent is efficacious in improving disease severity, reducing the risk of infection and reducing the signs and symptoms in patients 2 years old and older. It reduces the local inflammation in the skin and prevents further exacerbation of the disease with a good safety profile. Its structure contains a boron atom, which facilitates skin penetration and binding to the bimetal center of the phosphodiesterase 4 enzyme. It is currently under development as topical treatment of psoriasis (Paller et al., 2016).

Structure:



- **Chemical Formula:** C19H22FN3O3
- **Synonyms:** Crisaborole 906673-24-3, AN-2728, AN2728, 4-((1-Hydroxy-1,3 dihydro-benzo[c][1,2]oxaborol-5-yl)oxy)benzonitrile

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- **Molecular Weight:** 251.05 g/mol,Monoisotopic: 251.075373
- **IUPAC Name:** 4-[(1-hydroxy-3H-2,1-benzoxaborol-5-yl)oxy]benzonitrile

Pharmacodynamics: Crisaborole has broad-spectrum anti-inflammatory activity by mainly targeting phosphodiesterase 4 (PDE4) enzymes that is a key regulator of inflammatory cytokine production. As this enzyme is expressed in keratinocytes and immune cells, crisaborole mediates an anti-inflammatory effect on almost all inflammatory cells. Topical application of this drug is useful as it potentiates the localization of this drug in the skin and this anti-inflammatory activity is in the low micromolar range (Paton et al., 2017).

Mechanism of action: Inhibition of PDE4 by crisaborole leads to elevated levels of cyclic adenosine monophosphate (cAMP). Increased intracellular levels of cAMP inhibit the NF-kB pathway and suppress the release of pro inflammatory mediators such as TNF-alfa and various interleukins that play a causative role in psoriasis and atopic dermatitis. Suppression of downstream effects in different cell types may explain the therapeutic role of crisaborole in immune-mediated skin diseases (Zane et al., 2016).

Absorption: Systemic concentrations of crisaborole were reached by 8 days of twice daily topical administration. It has low systemic absorption thus poses less risk for developing systemic side effects.

Metabolism: Crisaborole is substantially metabolized into inactive metabolites. The major metabolite 5-(4-cyanophenoxy)-2-hydroxyl benzylalcohol (metabolite 1), is formed via hydrolysis; this metabolite is further metabolized into downstream metabolites, among which 5-(4 cyanophenoxy)-2-hydroxyl benzoic acid (metabolite 2), formed via oxidation, is also a major metabolite.

Chemical Taxonomy: This compound belongs to the class of organic compounds known as diarylethers. These are organic compounds containing the dialkyl ether functional group, with the formula ROR', where R and R' are aryl groups.

Toxicity: Hypersensitivity reactions such as contact urticaria may occur and discontinuation of the treatment is advised. No evidence of mutagenic or clastrogenic potential as well as altered effects on fertility. Oral LD50 value for rats is >500mg/kg (Moustafa et al., 2014).

MATERIAL AND METHOD

The Materials and Methods section of any research paper gives a full description of the tools, substances, and processes used to conduct the study. It contains information about the materials, such as chemicals, equipment, and biological samples, as well as a step-by-step explanation of the methods used during testing or data collecting. This section is critical for ensuring that the research is reproducible, allowing other scientists to accurately comprehend and repeat the findings. It lays the groundwork for the validity and trustworthiness of the research results.

Chemical and Glassware's required

List of chemicals

Table 1: List of chemicals

S. No	Reagents & Chemicals	Company
1	Crisaborole	Pfizer India
2	Cholesterol	Oxford
3	Soy lecithin	Himedia
4	KBr	Labogens
5	Liquid paraffin	Recombigen
6	Chloroform	Renkem
7	DMSO	Renkem
8	Ethanol	Analytical
9	Methanol	Rankem

List of glassware's

Table 2: List of glassware's

S. No	List of Glassware's	Type/Company
1	Test tubes	Borocil
2	Petri dishes	Borocil
3	Glass rod	Borocil
4	Beaker	Borocil
5	Conical flask	Borocil
6	Measuring cylinder	Borocil
7	glass slide	Borocil
8	Volumetric flask	Borocil

List of instruments

Table 3: List of instruments

S. No	Instruments	Model
1	Boiling water bath	Universal
2	Magnetic stirring	Remi
3	Weighing Machine	Sirtech
4	UV-Visible Spectrophotometer	Shimadzu 1700
5	Vortex shaker	Scientech (SE-146)
6	Melting point apparatus (Tempo)	Tempo
7	FTIR (Fourier-transform infrared spectroscopy)	Perkin Elmer Spectrum BX
8	Hot air oven	Scientech
9	Lyophilizer	Laborax
10	Zeta sizer	Hitachi High-Tech

11	Digital pH meter	Ei
12	dialysis bag	Aldrich

Pre-formulation studies

Pre-formulation studies of the drug were carried out to assess its fundamental physical and chemical properties before developing the final formulation. These studies included evaluations of solubility, melting point, pH, stability, and drug-excipient compatibility. Techniques such as UV spectroscopy and FTIR were used to analyze the drug's purity and interactions. The information obtained helped in understanding the drug's behavior, guiding the selection of suitable excipients, and ensuring the stability and effectiveness of the final product (Vilegave et al., 2013).

1 **Organoleptic Properties:** Crisaborole was observed as a white to off-white, odorless drug with a slightly bitter taste and smooth texture, meeting basic quality requirements (Fantini et al., 2020).

2 **Solubility study:** Solubility was assessed by dissolving the drug in various solvents. Clear solutions indicated good solubility, while turbidity or undissolved particles indicated poor solubility (Ansari et al., 2022).

3 **Melting Point:** The melting point was determined using a capillary method. The temperature at which the drug completely melted was recorded and compared with standard values to confirm purity (Chaudhary, 2020).

4 **pH determination:** The pH of the drug solution was measured using a calibrated digital pH meter to determine its acidic or basic nature, important for formulation stability (Awan et al., 2022)

5 Determination of Maximum Wavelength (λmax)

- **Preparation of Crisaborole standard stock solution in methanol:** Standard solutions of Crisaborole were prepared in methanol through serial dilution (Singh et al., 2020).
- **Lambda max:** The solution was scanned in the UV range (200–400 nm) to identify the absorption maximum (Kumbhar and Salunkhe 2013).
- **Linearity and Calibration Curve:** Dilutions (2–12 µg/mL) were analyzed at 250 nm, and a calibration curve of absorbance vs. concentration was plotted (Singh et al., 2020).

1 **Functional group identified by FTIR:** FTIR spectroscopy was used to identify functional groups and confirm molecular structure. The sample was prepared using the KBr pellet method and scanned in the range of 400–4000 cm^{-1} to obtain the characteristic spectrum (Lakshminarayanan and Balakrishnan 2020).

Preparation of Liposomes formulation

The liposomes were prepared by thin film hydration technique by using Rotary Evaporator instrument. The different formulation of Crisaborole loaded liposomes was prepared as shown in table no.1. Crisaborole (200 mg), Soya lecithin (200mg, 300mg, 400 mg, 500 mg and 600 mg) and Cholesterol (50 mg, 100 mg, 150 mg, 200 mg and 250 mg) was dissolved in 8 ml of Chloroform and 2 ml of ethanol. This solution was taken in a 250 ml round bottom flask. The flask was rotated in rotary flash evaporator at 80 rpm for 30 minutes in thermostatically controlled water bath at 40°C under vacuum 900 mm Hg. The organic solvent was slowly removed by this process such that a very thin film of dry lipids was formed on the inner surface of the flask. The dry lipid film was slowly hydrated with 10 ml of phosphate buffer pH 6.8 and rotated for 2 hr. swirling the contents to yield milky white suspension. The formulation is subjected to centrifugation at 3000 rpm for a period of 30 minutes. The liposome of Crisaborole was obtained & further it has been considering for its characterization and stored in well closed container for further use (Lonkar and Shinde et al., 2016, Moghimipour et al., 2013).

Table 4: Composition of Liposome formulation

S. No	Ingredients	Formulations				
		LSF1	LSF2	LSF3	LSF4	LSF5
1	Drug (mg)	200	200	200	200	200
2	Soya lecithin (mg)	200	300	400	500	600
3	Cholesterol (mg)	50	100	150	200	250
4	Phosphate buffer pH (6.8) (ml)	10	10	10	10	10
5	Chloroform (ml)	8	8	8	8	8
6	Ethanol (ml)	2	2	2	2	2
7	Methyl paraben (%)	0.02 %	0.02 %	0.02 %	0.02 %	0.02 %
8	Propyl papaben (%)	0.02 %	0.02 %	0.02 %	0.02 %	0.02 %

Evaluation parameter of liposome formulation

1 **Size Distribution:** Particle size, a key parameter for liposome characterization, was measured using

a Malvern Zeta Sizer. Samples were diluted with Millipore-filtered water and analyzed at 25°C to obtain size distribution data (Singh and Vingkar 2008).

2 **Zeta potential:** Zeta potential was measured to determine particle charge and stability. Liposome samples were diluted with distilled water, sonicated for 5–15 minutes, and analyzed using a Zetasizer (Đorđević et al., 2022).

3 **Scanning Electron Microscopic (SEM):** SEM was used to study the morphology of liposomes. Samples were coated with a thin metal layer (gold/palladium/platinum) and examined under an electron beam to obtain surface topography images (Anwer et al., 2019).

4 **Entrapment efficiency:** Entrapment efficiency was determined by mixing liposome dispersion with methanol, followed by filtration and dilution. The drug concentration was measured spectrophotometrically at 250 nm (Swetha et al., 2011).

Loading efficiency = Actual drug content in liposome / Theoretical drug content × 100

5 **In-vitro drug release:** In-vitro drug release was evaluated using the dialysis bag diffusion method in pH 7.4 phosphate buffer at 37 ± 2°C with constant stirring. Samples were withdrawn at regular intervals and analyzed at 250 nm using a UV-Visible spectrophotometer. Drug release data were analyzed using kinetic models such as zero-order, first-order, and Higuchi models to understand release behavior.

- Zero - order kinetic model – Cumulative % drug released versus time.
- First – order kinetic model – Log cumulative percent drug remaining versus time.
- Higuchi's model – Cumulative percent drug released versus square root of time.
- Korsmeyer-Peppas model - log cumulative % drug release vs log time (Kors- meyer–Peppas model)

Zero order kinetics

$$A_t = Ao\text{-}KoT$$

Zero order release would be predicted by the following equation:

Where, At=Drug release at time't', A0=Initial drug concentration, K0=Zero-order rate constant (hr-1)

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys Zero– order kinetics and its slope is equal to Zero order release constant K0.

First order kinetics

$$LogC=logC_o\text{-}K_t/\ 2.303$$

First-order release could be predicted by the following equation:

Where, C=Amount of drug remained at time't'; C0=Initial amount of drug; K=First-order rate constant (hr-1).

When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant 'K1'can be obtained When the data plotted as log cumulative percent drug remaining versus time, yields a straight line, indicating that the release follow first order kinetics. The constant 'K1' can be obtained by multiplying 2.303 with the slope value.

Higuchi's Model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q=[\ D\in/\tau(2A\text{-}\in Cs)\ Cst]^{1/2}$$

Where, Q= Amount of drug release at time't'; D= Diffusion coefficient of the drug in the matrix; A= Total amount of drug in unit volume of matrix; Cs= Solubility of drug in the matrix; Є= Porosity of the matrix; τ= Tortuosity; t= Time (hrs at which q amount of drug is released.

Above equation can be simplified as if we assume, that 'D', 'Cs' and 'A' are constant. Then equation: becomes.

Korsmeyer-Peppas model

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation (5). To find out the mechanism of drug release.

$$Mt\ /\ M\infty=Kt^n.$$

Where, Mt / M∞ are a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices.

Stability studies

The Crisaborole-loaded liposome formulation was packaged and placed in the stability test chamber and submitted to stability tests at accelerated testing (250C±20C and 60±5% RH) and (400C±20C and 70±5% RH) for 3 months. The formulation was tested for particle size evaluation and entrapment efficiency investigations at intervals of 30, 45, 60, and 90 days (3 months). According to International Conference on Harmonization (ICH) criteria, the formulation was tested for stability throughout a three-month period of accelerated storage. Formulation was investigated for changes in assessment parameter particle size and entrapment efficiency tests. All results were compared to the final formulation of 0 days as a reference (Sharma et al., 2014).

Anti-microbial activity

Preparation of Nutrient Agar Media: 2.8 g of Nutrient Media was dissolved in 100 ml of distilled water. The media's pH was checked prior to sterilization. The medium was autoclaved at 121 ˚C and 15 pounds of pressure for 15 minutes. Nutrient media was poured onto plates and placed under laminar air flow until the agar solidified.

Culture inoculation and well preparation: Use a sterilized cork-borer to make four wells in both agar plates. Lawn culture of bacteria E. coli and S. aureus was dispersed on Nutrient Agar Media with a spreader. Then, add the drug loaded liposome to the wells. The plate is incubated at 37 °C for 24 hours. Following incubation, the zone of inhibition around each well is determined. A bigger zone indicates more antibacterial action, while the absence of a zone implies no antimicrobial effect (Fattal et al., 1991).

RESULTS AND DISCUSSIONS

Pre-formulation study of Crisaborole

Organoleptic evaluation

The drug sample was analyzed physical appearance and the parameter given below in table 5.

Table 5: Organoleptic evaluation of Crisaborole

S. No	Physical parameter	Observation
1	Color	White
2	Odor	Slight
3	State	Solid
4	Appearance	Crystalline powder

Crisaborole's organoleptic properties, including color, odor, condition, and appearance, were examined. Crisaborole is a crystalline powder with a white, according to research. Crisaborole met the I.P. requirements for color, condition, odor, and appearance. Table 5 shows the outcomes.

Solubility study

Table 6: Solubility study of Crisaborole

Drug	Solvents	Observation/Inference
Crisaborole	Water	Insoluble
	Ethanol	Soluble
	Methanol	Freely Soluble
	Acetone	Soluble
	DMSO	Sparingly Soluble

Crisaborole solubility is shown in Table 6 in a variety of non-volatile or volatile liquid carriers, such as acetone, methanol, ethanol, DMSO, and water. Crisaborole is found to be easily soluble in methanol, soluble in ethanol and acetone, insoluble in water, and sparingly soluble in DMSO.

Melting Point

Table 7: Melting Point of Crisaborole

Drugs	Observed	Reference
Crisaborole	131°C	128.8 to 134.6 °C

Use a melting point equipment to determine the melting point of a substance. Crisaborole has a melting point of 131˚C, which meets the medication criteria.

Determination of pH

Table 8: pH determination

S. No	Drug	Observed
1	Crisaborole	5.3

A digital pH meter is used to determine a substance's pH. The pH of Crisaborole was discovered to be 5.3, which falls well within the drug's standard.

Determination of λ max by UV spectroscopy

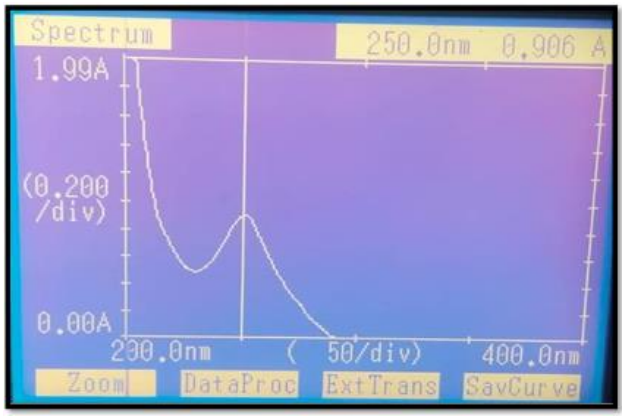


Figure 1: UV graph of Crisaborole (250 nm)

Standard calibration curve

Table 9: Calibration Curve of Crisaborole in Methanol

S. No	Concentration (µg/ml)	Mean Absorbance
1	2	0.179
2	4	0.354
3.	6	0.549
4	8	0.711
5	10	0.886
6	12	0.999
Mean	0.631	
SD	0.313899	
%RSD	49.746	

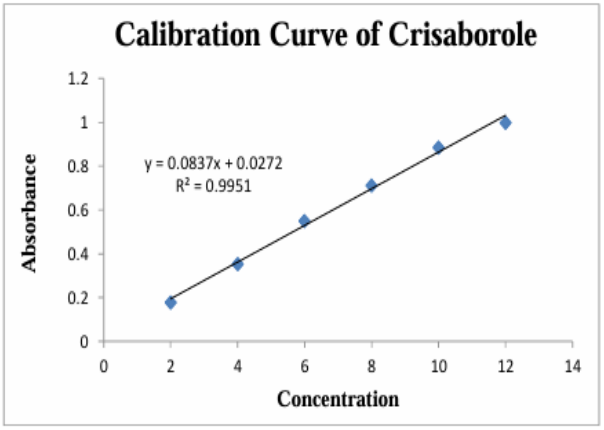


Figure 2: Calibration curve of Crisaborole

Discussion: The proposed method's linearity was proven through a calibration curve analysis utilizing least squares linear regression. The regression equation was developed by graphing the absorbance versus Crisaborole concentration from 2 to 12µg/ml. A 6-point calibration curve was generated for drug concentrations from 2 to 12 µg/ml. The drug's reaction was linear in the concentration range investigated, as indicated by the linear regression equation y = 0.0837x + 0.0272 and the correlation coefficient R^2 = 0.9951

Functional group identified by Infra-Red spectroscopy

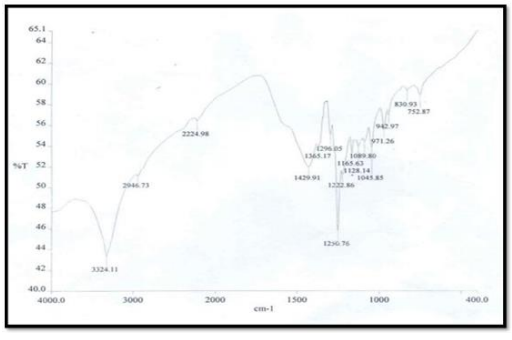


Figure 3: FTIR study of Crisaborole

Table 10: Interpretation of IR spectrum of Crisaborole

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3324.11	3550 – 3200	O-H stretch	Alcohols**
2	2946.73	2990 – 2850	C-H stretch	Alkanes
3	2224.98	2280 – 2200	C=N stretch	Nitriles
4	1429. 91	1440-1395	O-H bending	Carboxylic acid
5	1365.17	1400-1300	NO2 stretch	Nitro group
6	1296.71	1310-1250	C-O stretching	Aromatic ester
7	1128.14	1150-1085	C-O stretching	Aliphatic ether
8	1045.85	1050-1040	CO-O-CO stretching	Anhydride
9	942.97	950–910	O–H bend	Carboxylic acids
10	752.87	850–550	C–Cl stretch	Alkyl halides

FTIR spectra of Crisaborole represent Alcohols**, O-H stretch; broad peak appeared at 3324.11cm-1, C-H stretch peaks of Alkane at 2946.73. The C=N stretch peak of Nitriles at 2224.98cm-1, O-H bending peak Carboxylic acid at 1429. 91cm-1, NO^2 stretch peak of Nitro group at1365.17cm-1,C-O stretching peak of Aromatic ester at 1296.71cm-1.C-O stretching peak of Aliphatic ether at 1128.14 The CO-O-CO stretching peak of Anhydride at 1045.85 cm-1, O–H bend peak Carboxylic acids at 942.97cm-1, The C–Cl stretch peak of Alkyl halides at 752.87cm-1.

Characterization of liposome formulation

Particle size determination

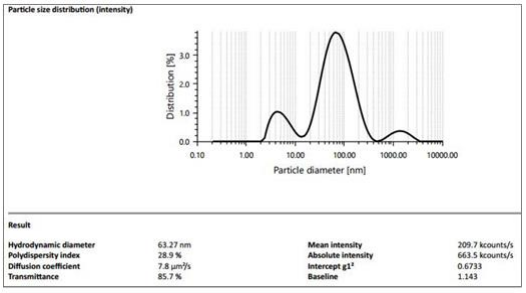
Table 11: Result of Particle size of all formulations

S. No	Formulations	Particle size (nm)	PI Value
1	Liposome (LSF 1)	63.27 nm	28.9
2	Liposome (LSF 2)	72.14 nm	27.7
3	Liposome (LSF 3)	43.07 nm	27.0
4	Liposome (LSF 4)	50.08 nm	30.0
5	Liposome (LSF 5)	75.17 nm	23.4

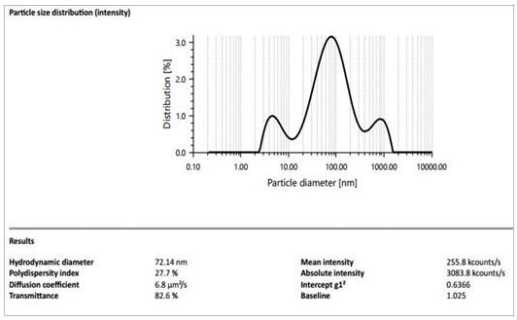
Particle size is a crucial factor in determining the characterisation of liposomes. The produced Liposome formulation's average particle size was measured using a Malvern zeta sizer. Particle size study revealed that the average particle size of liposomes ranged from 43.07 to 75.17 nm. These particle size results demonstrate that all formulated Liposomes fall inside the Liposome range, with

F3 having the smallest particle size of all formulations reported in table 11.

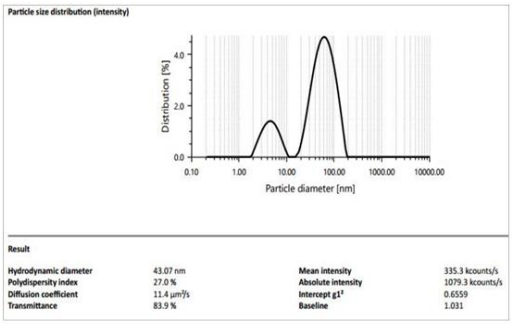
LSF1



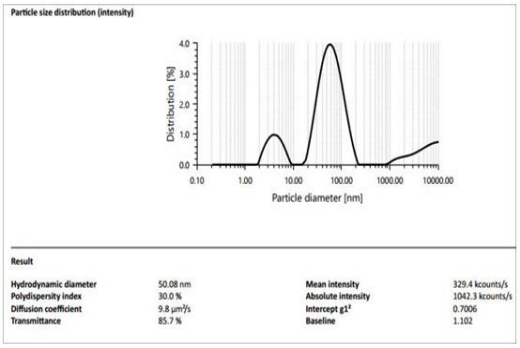
LSF2



LSF3



LSF4



LSF5

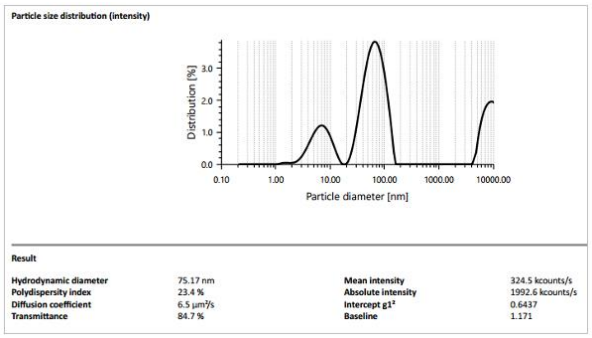


Figure 4: Particle size (LSF1-LSF5)

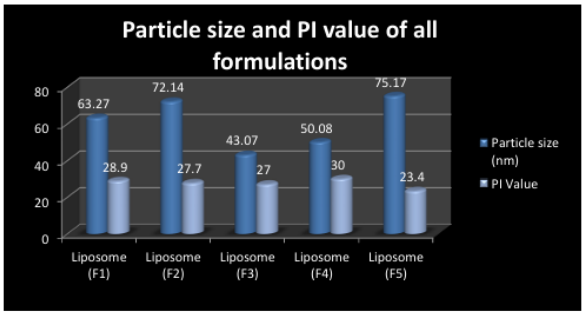
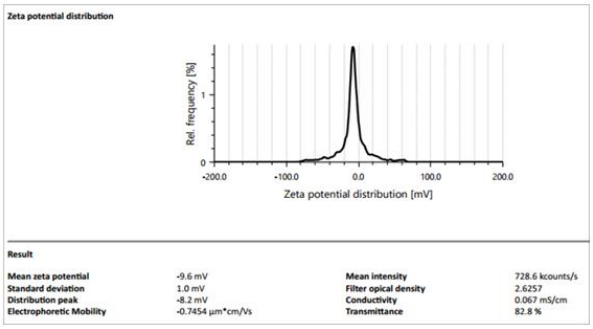


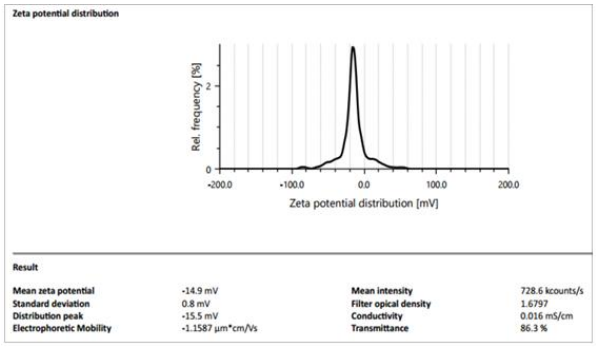
Figure 5: Particle size and PI value of all formulations

Zeta potential Analysis

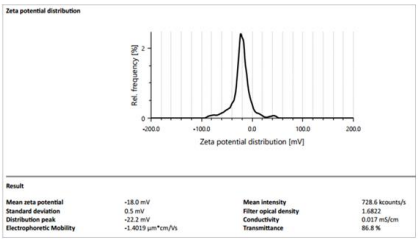
LSF1



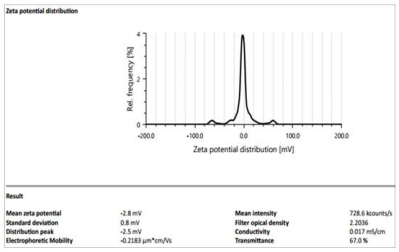
LSF2



LSF3



LSF4



LSF5

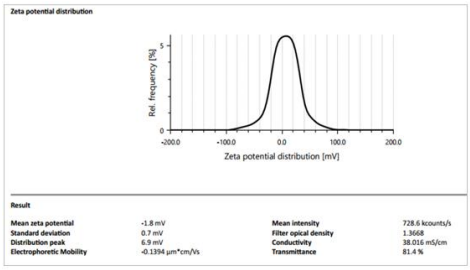


Figure 6: Zeta potential (LSF1- LSF5)

Table 12: Result of Zeta potential of all formulations

S. No	Formulation	Zeta potential
1	Liposome (LSF 1)	-9.6 mV
2	Liposome (LSF 2)	-14.9 mV
3	Liposome (LSF 3)	-18.0 mV
4	Liposome (LSF 4)	-2.8 mV
5	Liposome (LSF 5)	-1.8 mV

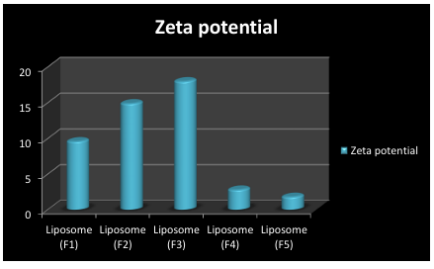


Figure 7: Zeta potential graphical representations

To determine the particles' surface charge, a zeta potential examination is performed. The magnitude of the zeta potential predicts the colloidal stability. The zeta potential ranged from 1.8 to -18.0 mV across all formulations, with a peak area of 100% intensity. These numbers suggest that the Liposome is stable throughout its formulation. The results are shown in above Table.

Scanning electron microscopy characterization of LSF3 formulation

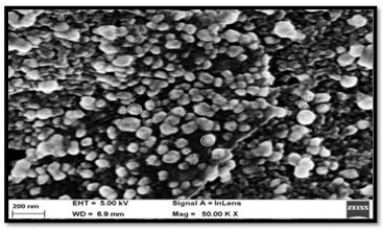


Figure 8: SEM

SEM analysis was used to determine the microscopic characteristics (shape and morphology) of the produced liposome. Drug-loaded liposomes were manufactured and thoroughly dried to reduce moisture content before being imaged using scanning electron microscopy. A scanning electron micrograph of the prepared Liposome at 50.00 kx magnification revealed that the Liposome had a smooth surface morphology and a sphere form. The SEM pictures clearly showed the smooth surface morphology and spherical shape of the Liposomes. Figure 23 illustrates this.

Entrapment efficacy

Table 13: Entrapment efficacy

S. No	Formulations (LSF1-LSF 5)	Entrapment efficacy (%)
1	Liposome (LSF 1)	73.43
2	Liposome (LSF 2)	81.57
3.	Liposome (LSF 3)	93.48
4	Liposome (LSF 4)	86.34
5	Liposome (LSF 5)	79.68

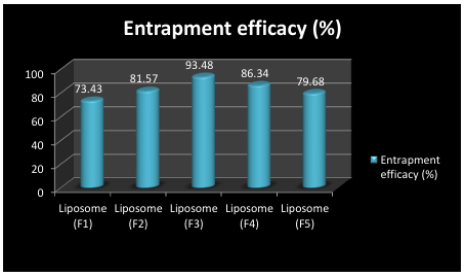


Figure 9: Entrapment efficacy graphical representations

This could be because the variance in entrapment efficiency was caused by differences in polymer concentration and varying degrees of crosslinking.

The prepared liposome has a drug entrapment range of 73.43 to 93.48. The LSF3 Liposome has a high drug entrapment efficiency, which was reported to be at 93.48%.

Table 14: Release studies of all formulations

S. No	Time (Hr)	cumulative % drug released LSF1	cumulative % drug released LSF 2	cumulative % drug released LSF 3	cumulative % drug released LSF 4	cumulative % drug released LSF 5
1	0	0	0	0	0	0
2	2	13.25	20.18	14.97	11.17	20.55
3	3	28.14	31.47	19.27	28.46	35.47
4	4	39.13	46.74	29.22	33.36	42.65
5	5	50.41	57.07	48.45	50.10	53.16
6	6	68.72	66.83	60.75	67.28	62.30
7	7	89.23	75.28	79.46	79.11	77.36
8	8	92.23	84.13	84.83	88.38	81.72
9	9	94.28	90.07	96.77	92.39	89.27

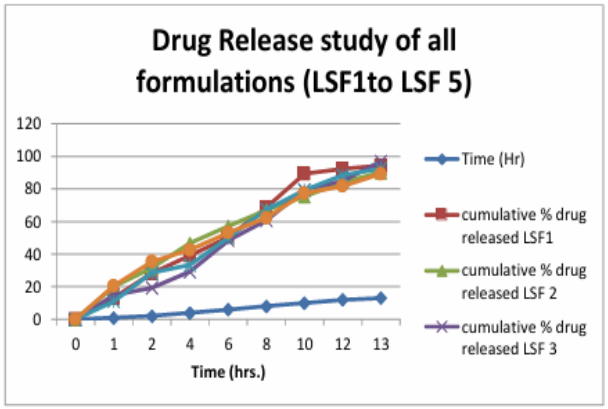


Figure 10: Release studies of all formulations

In-vitro drug release

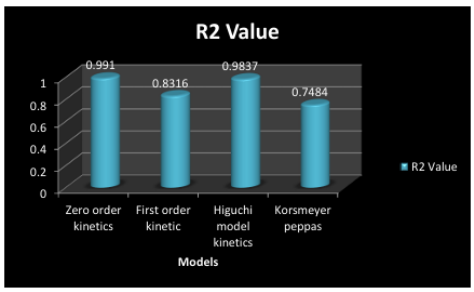
Table 15: Release kinetics study of LSF3 Liposome formulation

Time Hr	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
1	14.97	85.03	1.000	1.930	0.000	1.175
2	19.27	80.73	1.414	1.907	0.301	1.285
4	29.22	70.78	2.000	1.850	0.602	1.466
6	48.45	51.55	2.449	1.712	0.778	1.685
8	60.75	39.25	2.828	1.594	0.903	1.784
10	79.46	20.54	3.162	1.313	1.000	1.900
12	84.83	15.17	3.464	1.181	1.079	1.929
13	96.77	3.23	3.606	0.509	1.114	1.986

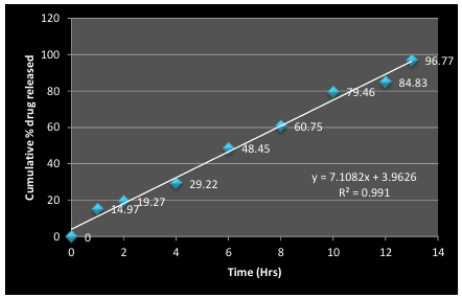
Table 16: Regression coefficient (R2 Value)

S. No	Models	R2 Value
1	Zero order kinetics	0.9910
2	First order kinetic	0.8316
3	Higuchi model kinetics	0.9837
4	Korsmeyer peppas	0.7484

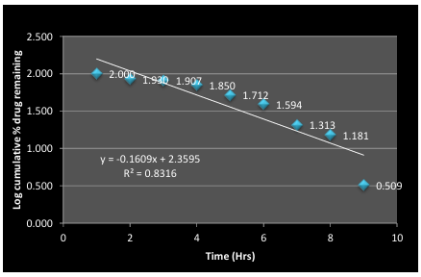
(a)



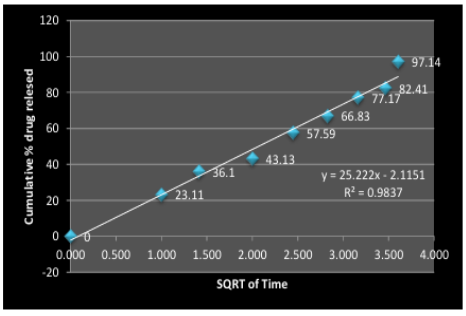
(b)



(c)



(d)



(e)

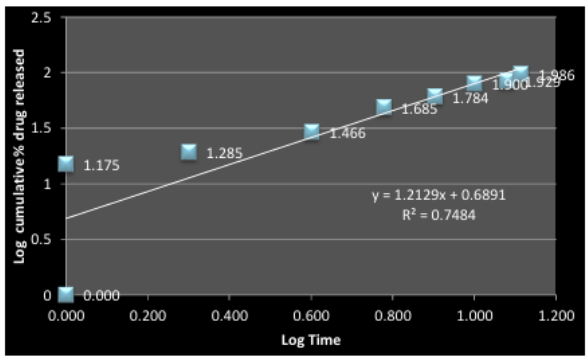


Figure 11: Graphical representation of (a) Release kinetics study; (b) Zero Order; (c) First order; (d) Higuchi Model; (e) Korsmeyer peppas.

The following plots were created for the kinetic study: cumulative% drug release vs. time (zero order kinetic models); log cumulative% drug remaining vs. time (first order kinetic model); cumulative% drug release vs. square root of time (Higuchi model); and log cumulative% drug release vs log time (Korsmeyer-Peppas model). Zero order kinetic models describe the mechanism of constant drug release from a drug delivery device that is independent of concentration. The modified formulation's zero order graph showed constant drug release from the Liposome, with the zero order model yielding y = 7.1082x + 3.9626, R2 = 0.991. The first order kinetic model depicts a system's release rate as a function of concentration. The first-order kinetic model resulted in y = -0.1609x + 2.3595, R^2 = 0.8316. The Higuchi model defines the boundaries of drug transport and release. The Higuchi formulation model was revealed to be: y = 25.222x - 2.1151, R^2 = 0.9837. The Korsmeyer-Peppas kinetic model resulted in y = 1.2129x + 0.6891, R^2 = 0.7484. Drug release experiments in vitro were performed utilizing the dialysis bag method. R2 represents the correlation value in the table above. Based on the best fit with the highest correlation (R2) value, it is decided that the Zero kinetic model should be used in the F3 Liposome formulation.

Stability study

Table 17: Stability Study of optimized formulation (Liposome)

S. No	Time (Days)	250C±2 0C and 60 ± 5% RH		400C±2 0C and 70 ±5% RH	
		Particle size	Entrapment efficacy (%)	Particle size	Entrapment efficacy (%)
1	0	43.07 nm	93.48 %	43.07 nm	93.48 %
2	30	42.33 nm	94.12 %	41.97 nm	94. 32 %
3	45	40.62 nm	94.89%	42.43 nm	93.86 %
4	60	43.89 nm	93.84%	40.54 nm	94.74 %
5	90	42.77 nm	95.84%	43.90 nm	93.14%

The formulation remained physically and chemically stable for three months under accelerated stability conditions (250C±20C and 60 ± 5% RH) and (400C±20C and 70 ± 5% RH). The evaluation parameters, including particle size and entrapment efficacy, did not change appreciably. Table 17 summarizes the assay results and other evaluation criteria at periodic time points in stability investigations. The above table shows the results of accelerated stability experiments on Liposome formulations. No significant changes were found.

Antimicrobial activity of Liposome Formulation against E.coli and S.aureus

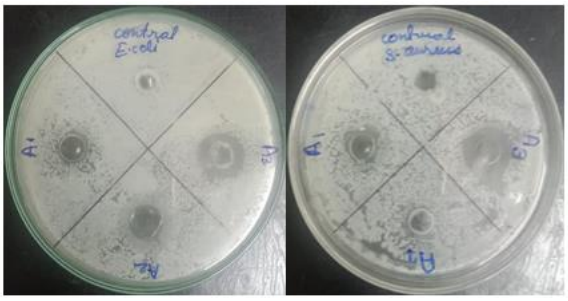


Figure 12: Antimicrobial activity against E.coli and S.aureus

Table 18: Antimicrobial activity of Liposome Formulation against E.coli and S.aureus

S. No.	Sample Name (mg/ml)	Zone of Inhibition (mm) of E.coli	Zone of Inhibition (mm) of S.aureus
1	(Control)	0 mm	0 mm
2	A1 (2 mg/ml)	2.0 mm	4.0 mm
3	A 2 (4 mg/ml)	5.0 mm	7.0 mm
4	A 3 (8 mg/ml)	10.0 mm	13.0 mm

This data indicates that the plant extract loaded Liposome formulation has a stronger antibacterial effect against E.coli compared to the S.aureus bacteria.

SUMMARY

The research aimed to design, formulate, develop, and optimize a novel liposomal vesicle system containing crisaborole using the thin-film hydration method with soya lecithin. This was done to enhance drug solubility, improve

dermal delivery, and evaluate the antibacterial performance in comparison to the pure drug. The melting point was observed at 1310C, and the pH of the drug solution was 5.3. UV spectrophotometric analysis identified λ max at 250 nm, and the calibration curve showed linearity in the concentration range of 2–12 µg/mL with an R^2 value of 0.9951. Characterization of the liposomes revealed an average particle size of 43.07 nm, a zeta potential of -18.0 mV, and a poly dispersity index (PDI) of 27.0, indicating uniform distribution. SEM images confirmed spherical morphology with smooth surfaces. Entrapment efficiency was found to be 93.48 %, Kinetic model followed the Zero order kinetics model (R^2 = 0.991), suggesting diffusion-controlled release. Stability studies conducted over 30 days at refrigerated conditions showed minimal changes in particle size and entrapment efficiency, confirming formulation stability. The antimicrobial activity assessed via well diffusion assay showed that liposome crisaborole produced an average zone of inhibition of 13 mm against Staphylococcus aureus and 10 mm against E. coli, respectively, indicating enhanced antibacterial activity of the liposomal formulation.

CONCLUSION

The study successfully demonstrated that the liposomal vesicle system containing crisaborole, developed through the thin-film hydration technique, significantly improved the physicochemical and therapeutic properties of the drug. The optimized formulation exhibited favorable solubility, appropriate pH, and confirmed functional group compatibility. The small and uniform particle size, high entrapment efficiency, and stable zeta potential indicated an effective and stable delivery system. The in vitro drug release profile followed Higuchi kinetics, suggesting a diffusion-driven mechanism suitable for sustained topical delivery. Furthermore, the well diffusion assay clearly revealed that the liposomal formulation had superior antibacterial efficacy compared to the free drug, particularly against both gram-positive and gram-negative bacterial strains. These findings support the potential of liposomes crisaborole for improved dermatological applications, with enhanced drug delivery and antibacterial performance. The research not only established a successful formulation approach but also opened prospects for future studies focusing on clinical evaluation, incorporation into hydrogel bases, and use in combination therapies for resistant skin infections.

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