



Formulation and Evaluation of Baclofen Loaded Bilosomes as Topical Gel

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ABSTRACT

This study aimed to develop Baclofen -loaded bilosomes (BBS) using thin-film hydration followed by ultrasonication to improve solubility, drug delivery, antifungal activity, and stability. An (23) factorial design was employed to optimize eleven formulations using lipids, sodium deoxycholate, and Span 60, with particle size and entrapment efficiency as responses. The optimized formulation (BBS-OPT) exhibited a particle size of 206.21 nm, PDI of 0.258, zeta potential of -29.3 mV, entrapment efficiency of 81.17%, and drug content of 96.9%. FTIR and DSC confirmed drug-excipient compatibility, while SEM revealed uniform morphology. The developed baclosomal topical gel demonstrates significant potential as a novel and effective system for the controlled release of Baclofen, potentially enhancing therapeutic efficacy and patient compliance in the treatment of muscle spasticity.

Keywords: Baclofen, Bilosomes, Bile salt-stabilized vesicles, Antifungal activity.

1. Introduction

The skin is the largest organ of the human body, covering approximately 1.5–2.0 m² and accounting for about 16% of body weight. It provides a convenient route for local (dermal) and systemic (transdermal) drug delivery. Topical drug delivery offers advantages over oral and parenteral administration, including localized drug action, reduced systemic side effects, avoidance of first-pass metabolism, and improved patient compliance. However, the stratum corneum acts as the primary barrier to drug penetration, and most drugs lack the ideal physicochemical properties required for effective transdermal delivery.¹

Conventional topical formulations, such as creams, ointments, gels, and lotions, may exhibit inadequate skin retention and limited bioavailability. Therefore, novel drug delivery systems are being developed to enhance skin penetration and therapeutic efficacy. Nanocarriers, including liposomes, niosomes, lipid nanoparticles, and nanoemulsions, can improve drug penetration, increase skin retention, and provide controlled local drug delivery.²

Advantages of topical drug delivery system

- Avoids hepatic first-pass metabolism.
- Easy to use and apply.
- Medication can be stopped easily.
- Enables selective drug delivery to specific sites.
- Avoids gastrointestinal intolerance.
- Improves patient compliance.
- Allows self-medication.

Skin Structure

The skin is the body's first line of defense against external physical, mechanical, and chemical insults. It covers approximately 1.5–2.0 m² and accounts for about 16% of adult body weight. It also maintains homeostasis through thermoregulation and serves as an important site for local and systemic drug delivery due to the blood capillaries present in the dermis.

Human skin consists of three main layers: the epidermis, dermis, and hypodermis.

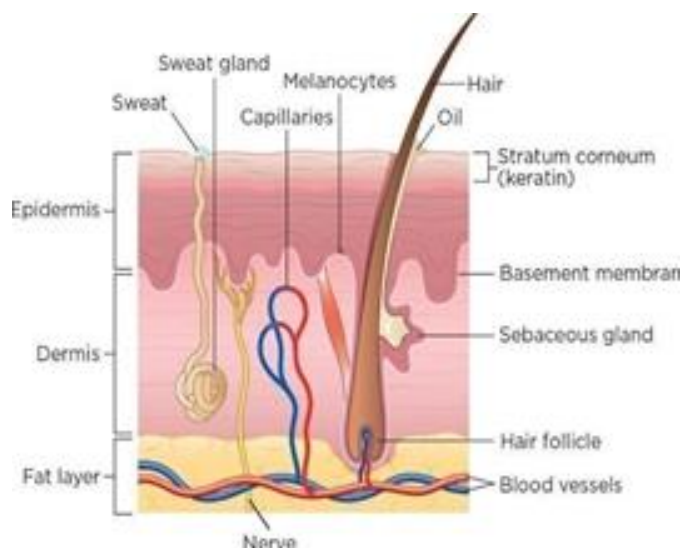


Figure-1: Anatomy of the Skin

Routes of Drug Penetration Through the Skin

The skin provides a large surface area for drug absorption; however, the stratum corneum (SC) acts as the primary barrier to drug penetration. Drugs cross the intact skin through two main pathways: **transepidermal and transappendageal pathways**.

The drug penetration through the intact skin via the stratum corneum can take place in two ways, namely the transepidermal and transappendageal pathways. In the transepidermal pathway, the drug is penetrated through the stratum corneum, and pathways can take place intra- or inter-cellular.²⁷ The intra-cellular route through corneocytes, terminally differentiated keratinocytes, allows the transport of hydrophilic or polar solutes. Transport via inter-cellular spaces allows diffusion of lipophilic or non-polar solutes through the continuous lipid matrix. The trans appendageal route involves the passage of molecules through sweat glands and across the hair follicles.²⁸

DISEASE PROFILE

Spasticity

Support Program for Assembly of a Database for Spasticity Measurement (SPASM) project defines spasticity as—disordered sensory-motor control, resulting from an [upper motor neuron lesion](#), presenting as intermittent or sustained involuntary activation of muscles. Spasticity is a common consequence of upper motor neuron syndrome. It occurs in several neurological conditions, such as stroke, cerebral palsy, multiple sclerosis, traumatic brain injuries, spinal cord injury and others. Spasticity has received considerable attention in literature due to its impacts on daily activities and movement. It might also lead to other comorbidities, such as joint contractures and deformities.²⁷

Baclofen has significant pharmacokinetic drawbacks when taken orally because it has a short biological half-life of 3–4 hours and is absorbed in the upper small intestine, making its duration of action limited. Patient failure to comply results from the requirement that it be taken often.²⁸ Recent studies attempted to develop oral dosage forms of sustained release in response to all the prior restrictions of oral baclofen, but the efforts failed for a variety of reasons, including dose dumping.²⁹

Spasticity is a common consequence of upper motor neuron syndrome associated with neurological disorders such as stroke, cerebral palsy, multiple sclerosis, traumatic brain injury, and spinal cord injury. Post-stroke spasticity affects approximately 30–80% of stroke survivors and commonly develops within the first one to three months. It predominantly affects upper-limb flexors and lower-limb extensors, impairing motor control and daily activities. Associated complications include pain, stiffness, joint contractures, and musculoskeletal deformities, which reduce quality of life and increase healthcare and caregiving needs. Early identification and appropriate management are essential to prevent complications, improve functional outcomes, and promote patient independence. Greater motor impairment, sensory deficits, and poor baseline functional status are important predictors of post-stroke spasticity.

BILOSOMES

Bilosomes are modified lipid-based vesicular carriers containing non-ionic surfactants and bile salts, which enhance drug solubility, absorption, and permeation. They are closed bilayer vesicles that may be unilamellar or multilamellar, generally spherical in shape, with reported sizes varying according to formulation. Bilosomes were first described by Conacher et al. in 2001. Bile salts help solubilize lipids by forming mixed micelles and can enhance the transport of drugs across biological barriers, including the intestinal mucosa, cornea, and skin. Therefore, bilosomes offer a promising approach for improving the delivery of poorly soluble and poorly permeable drugs.³⁰

Bilosomes Structure and Composition

Bilosomes are spherical, amphiphilic vesicles capable of encapsulating both hydrophilic drugs within their aqueous core and lipophilic drugs within their bilayer. They mainly consist of non-ionic surfactants, bile salts, phospholipids, and cholesterol.³¹

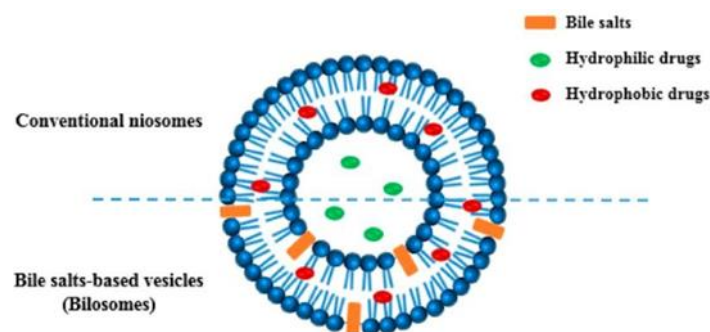


Figure 2: Structure of Bilosomes

Bilosomes consist of lipids, non-ionic surfactants, and bile salts. Hydrophilic drugs are generally entrapped within the aqueous core, while lipophilic drugs are incorporated into the bilayer.

1. Bile Salts: Bile salts are amphiphilic molecules that act as solubilizing agents and penetration enhancers by interacting with biological membranes. They can improve the absorption and bioavailability of poorly soluble or poorly permeable drugs. Common examples include sodium deoxycholate, sodium glycocholate, and sodium taurocholate.

2. Phospholipids: Phospholipids are biocompatible, amphiphilic molecules that self-assemble into bilayers in aqueous environments. They provide structural support and possess emulsifying properties. Common examples include soybean phosphatidylcholine, soya lecithin, and dicetyl phosphate.

3. Cholesterol: Cholesterol is incorporated into the bilayer to regulate membrane fluidity, improve vesicular stability, and increase membrane rigidity.

4. Non-ionic Surfactants: Non-ionic surfactants contribute to vesicle formation, solubilization, wetting, emulsification, and drug permeation. Common examples include sorbitan esters (Spans) and polysorbates (Tweens).

Advantages

1. **Enhanced Bioavailability:** Improves drug absorption and bioavailability.
2. **Targeted Delivery:** Enables targeted delivery to specific cells or tissues.
3. **Drug Protection:** Protects drugs from enzymatic and chemical degradation.
4. **Reduced Toxicity:** May reduce systemic toxicity and adverse effects.
5. **Gene Therapy:** Can deliver genetic material into cells.
6. **Biocompatibility:** Uses generally biocompatible lipids and bile salts.

Disadvantages

1. **Complex Preparation:** Requires specialized equipment and expertise.
2. **Stability Issues:** Sensitive to storage conditions and temperature.
3. **Immune Response:** May trigger immune reactions.
4. **High Cost:** Preparation and production can be expensive.
5. **Limited Applications:** Clinical applications remain limited.^{32, 34}

Methods of Preparation

1. Thin-film hydration technique
2. Solvent evaporation method
3. Reverse-phase evaporation method

4. Hot homogenization method
5. Ethanol injection method

i. Thin-Film Hydration Technique

The drug, cholesterol (CHO), surfactants, and phospholipids are dissolved in an organic solvent in a round-bottom flask. The solvent is evaporated under reduced pressure using a rotary evaporator to form a thin lipid film. The film is hydrated with distilled water or phosphate-buffered saline containing bile salts. The dispersion is sonicated to reduce vesicle size and stored at 4°C.

ii. Solvent Evaporation Method

The drug, surfactant, bile salt, and cholesterol are dissolved in an organic solvent. The solvent is evaporated using a magnetic stirrer, and the resulting residue is hydrated with phosphate buffer to obtain a bilosomal suspension.

iii. Reverse-Phase Evaporation Method

Soybean phosphatidylcholine and bile salts are dissolved in an organic solvent, followed by the gradual addition of an aqueous buffer. The mixture is sonicated to form a water-in-oil emulsion and evaporated under reduced pressure. The resulting material is hydrated with buffer, homogenized, and purified to obtain drug-loaded bilosomes.

iv. Hot Homogenization Method

Lipid components, such as monopalmitoyl glycerol, cholesterol, and dicetyl phosphate, are melted and hydrated with buffer. The mixture is homogenized, followed by bile salt addition and further homogenization. The antigen or drug is incorporated, and freeze–thaw cycles may be used to improve entrapment.

v. Ethanol Injection Method

The drug, surfactant, and cholesterol are dissolved in ethanol, while bile salts are dissolved in the aqueous phase. The ethanolic solution is injected into a larger volume of heated phosphate buffer under continuous stirring. After ethanol evaporation, the resulting dispersion is sonicated and stored at 4°C.

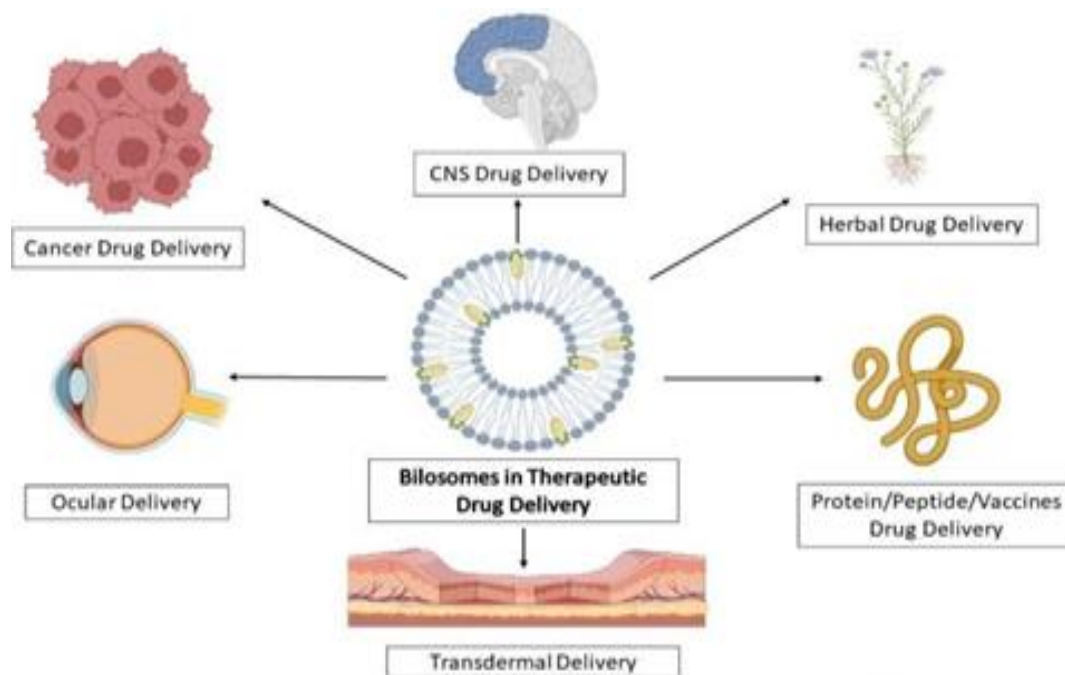


Figure 3: Applications of Bilosomes

Topical Gels

Topical gels are homogeneous semisolid preparations used to treat and prevent skin diseases. According to the USP, gels consist of small inorganic particles or large organic molecules dispersed in a liquid medium.

Gel Structure

Gels contain natural or synthetic polymers that form a three-dimensional network within a liquid medium. Gelling agents provide consistency and help retain the formulation on the skin, facilitating drug delivery.

Desirable Properties of Topical Gels

- Should contain inert and non-toxic ingredients.
- Should be non-sticky and easy to apply.
- Should not irritate the skin.
- Should be compatible with other additives.
- Should be easy to use and remove.
- Should contain suitable antimicrobial agents to prevent microbial growth.

Recent Approaches in Topical Gels

1. Emulgel: Emulgels combine emulsions with gels to improve the topical delivery of drugs, particularly poorly water-soluble drugs. They offer good spreadability, easy removal, improved stability, and a non-greasy appearance.

2. In-Situ Gel: In-situ gels are formulations that undergo a sol-to-gel transition under specific physiological conditions. They provide prolonged and controlled drug release while improving patient comfort and compliance.

3. Vesicle-Based Gel: Vesicle-based gels incorporate lipid- or surfactant-based vesicles, such as liposomes, niosomes, and bilosomes, into a gel matrix. These systems can improve drug penetration, skin retention, and controlled topical drug delivery.³⁵

Objectives

The aim of the present work is to formulate, optimize, and evaluate Baclofen -loaded bilosomes as a topical gel. The study includes preformulation studies of Baclofen and drug-excipient compatibility studies using FTIR and DSC. Baclofen-loaded bilosomes will be developed using the thin-film hydration method with a 2^3 factorial design and characterized for their physicochemical properties. The optimized bilosomal formulation will be incorporated into a topical gel and evaluated for its physicochemical properties, in-vitro drug release, and release kinetics. Comparative antifungal studies will be performed to assess antifungal activity, followed by short-term stability studies of the optimized bilosomal gel according to ICH guidelines.

METHODS

1. Preformulation Studies

Preformulation studies were conducted to assess the physicochemical properties of Baclofen

a. Organoleptic Properties: The colour and odour of Baclofen were examined visually and physically.

b. Solubility Studies: Excess Baclofen was added to ethanol, chloroform, acetone, methanol, and buffer solutions and shaken for 48 hours. The samples were filtered through Whatman filter paper No. 41 and analyzed spectrophotometrically.

c. Melting Point: The melting point of Baclofen was determined using Thiele's tube. The experiment was performed in triplicate, and the average value was recorded.

d. UV Spectrophotometric Analysis: Baclofen was analyzed using a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan).

Determination of λ_{max} : A diluted solution of Baclofen in phosphate buffer (pH 7.4) was scanned between 200 and 400 nm against a blank to determine the absorption maximum.

Preparation of Calibration Curve: A stock solution was prepared by dissolving 100 mg of Baclofen in 10 mL of ethanol and making up the volume to 100 mL with phosphate buffer (pH 7.4). Further dilutions were prepared to obtain concentrations of 5–25 $\mu\text{g/mL}$. Absorbance was measured at 298 nm, and a calibration curve was plotted against concentration. The method was evaluated for linearity, accuracy, and precision.

Preparation of Phosphate Buffer (pH 7.4): Potassium dihydrogen phosphate and sodium hydroxide solutions were prepared separately, and appropriate volumes were mixed and diluted with distilled water to obtain pH 7.4.

2. Drug-Excipient Compatibility Studies

a. Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectra of pure Baclofen and excipients were recorded using a BRUKER IR spectrophotometer over the range of 4000–600 cm^{-1} to assess compatibility.

b. Differential Scanning Calorimetry (DSC): DSC thermograms of pure drug, excipients, and optimized bilosomes were recorded using a DSC-60 (Shimadzu, Japan). Samples were heated from 0–300°C at 10°C/min under nitrogen purging at 50 mL/min.

3. Formulation of Baclofen -Loaded Bilosomes (LBS)

Baclofen -loaded bilosomes were prepared using the thin-film hydration method. Baclofen (50 mg), Span 60, cholesterol, lecithin (1:1 ratio), and sodium deoxycholate were dissolved in a chloroform–methanol mixture (2:1). The organic solvents were evaporated under vacuum at 60°C using a rotary evaporator to form a thin lipid film. The film was hydrated with 10 mL of phosphate buffer (pH 7.4) at 100 rpm for 1 hour. The resulting dispersion was sonicated for three 1-minute cycles, with 5-minute intervals. Finally, the formulation was refrigerated at 4°C overnight for vesicle maturation and stored until further evaluation.

4. Experimental Design

A (2³) factorial design was used to investigate the effects of three independent variables: lipid amount (X₁), sodium deoxycholate (SDC) amount (X₂), and Span 60 amount (X₃) on vesicle size and entrapment efficiency. Each factor was studied at two levels (–1 and +1), and 11 experimental batches were prepared. Multiple linear regression and Design-Expert® software (Version 12) were used to analyze the responses, evaluate model fit using ANOVA, and generate response plots. The optimized formulation was selected by minimizing vesicle size and maximizing entrapment efficiency, and its experimental responses were compared with predicted values

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + \dots$$

Factor	Low (–1)	High (+1)
Lipids (mg)	30	40
SDC (mg)	10	20
Span 60 (mg)	120	240

Table No. 1: Independent variables and their corresponding levels for Baclofen Loaded Bilosomes (LBS)

Formulation of Baclofen Loaded Bilosomes (LBS)

Table No 2: Formulation Table of Baclofen Loaded Bilosomes (LBS) as per

the runs given by 23 factorial study

Formulation	X1, Lipids (mg)	X2, SDC (mg)	X3 , Span 60 (mg)	X1, Lipids (mg)	X2, SDC (mg)	X3, Span 60 (mg)
Code	Levels			Actual amount		
F1	0	0	0	35	15	180
F2	+1	+1	–1	40	20	120
F3	0	0	0	35	15	180
F4	0	0	0	35	15	180
F5	–1	+1	–1	30	20	120
F6	–1	–1	+1	30	10	240
F7	–1	–1	–1	30	10	120
F8	+1	–1	–1	40	10	120
F9	+1	+1	+1	40	20	240
F10	+1	–1	+1	40	10	240
F11	–1	+1	+1	30	20	240

50 mg of pure drug Baclofen and organic solvents are kept constant for all the formulations.

5. Characterization of Baclofen -Loaded Bilosomes

a. Particle Size, PDI and Zeta Potential: Vesicle size, polydispersity index (PDI), and zeta potential were measured using a Malvern Zetasizer to assess particle size distribution and physical stability.

b. Scanning Electron Microscopy (SEM): Surface morphology of the optimized bilosomes was examined using a ZEISS EVO LS15 scanning electron microscope.

c. Entrapment Efficiency (%EE): The bilosomal dispersion was centrifuged at 10,000 rpm for 30 minutes at 10°C. The supernatant was diluted with phosphate buffer (pH 7.4), and free drug was measured at 298 nm using a UV-visible spectrophotometer.

d. In-vitro Drug Release: Drug release was studied using a Franz diffusion cell with a 1.76 cm² diffusion area and 20 mL receptor compartment containing phosphate buffer (pH 7.4). One gram of bilosomal dispersion was placed on a dialysis membrane and maintained at 37°C with stirring at 100 rpm. Samples were withdrawn at predetermined intervals, replaced with fresh buffer, and analyzed at 298 nm for 24 hours to calculate cumulative drug release.

6. Preparation of Baclofen -Loaded Bilosomal Gel

Carbopol 940 (1% w/v) was dispersed in water and allowed to swell overnight. The optimized bilosomal dispersion was incorporated with continuous stirring. Triethanolamine (0.5%), methylparaben, and propylparaben (0.1%) were added, and the pH was adjusted. The prepared gel was stored for further evaluation.

7. Evaluation of Baclofen -Loaded Bilosomal Gel

i. Appearance and Homogeneity: Colour, odour, and consistency were examined visually and by spreading a small quantity of gel between the fingers.

ii. pH: The pH was measured using a calibrated pH meter, and the average of three readings was recorded.

iii. Spreadability: One gram of gel was placed between two glass plates, loaded with a 125 g weight for one minute, and the spread diameter was measured.

iv. Viscosity: Viscosity was measured using a Brookfield DVE viscometer at 25°C with spindle No. 64 at 50–250 rpm.

v. Drug Content: The bilosomal gel sample was diluted with phosphate buffer (pH 7.4), centrifuged, and analyzed spectrophotometrically at 298 nm. Drug content was calculated as the percentage of measured drug relative to the total drug added.

vi. Extrudability: Gel was packed in collapsible aluminium tubes, and the amount extruded under a 500 g weight was measured. Extrudability was classified as excellent (>90%), good (>80%), or fair (approximately 70%).

vii. In-vitro Drug Release: Drug release was evaluated using a Franz diffusion cell with a dialysis membrane, phosphate buffer (pH 7.4), and UV analysis at 298 nm over 24 hours.

viii. Drug Release Kinetics: Release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The best-fit model was identified using the correlation coefficient (R^2) and root mean square error (RMSE) with DD-Solver software.

8. Irritation Study

The irritation potential of the bilosomal gel was evaluated using the hen's egg chorioallantoic membrane (HET-CAM) test. Fertilized eggs were incubated for 10 days at $37 \pm 0.5^\circ\text{C}$ and $60 \pm 1\%$ relative humidity. The test formulation, 1% sodium lauryl sulphate (positive control), and 0.9% sodium chloride (negative control) were applied to the CAM and observed for up to five minutes for haemorrhage, vessel lysis, and membrane discoloration.

9. Antifungal Studies

The antifungal activity of pure Baclofen, bilosomes, bilosomal gel, and marketed cream was evaluated against *Candida albicans* and *Aspergillus niger* using the agar well-diffusion method. Sabouraud dextrose agar plates were inoculated with the fungal cultures, and samples were added to 6 mm wells. After incubation for 72 hours, zones of inhibition were measured and compared.

10. Short-Term Stability Studies

The bilosomal gel was packed in collapsible aluminium tubes and stored at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity for 90 days, according to the stated ICH conditions. After storage, the formulation was evaluated for pH, spreadability, extrudability, viscosity, and *in-vitro* drug release.

RESULTS

1. Preformulation studies:

a. Organoleptic properties

Table no 3: Organoleptic properties of Baclofen

Sl. No	Parameters	Observation
1	Nature	Crystalline
2	Color	White to yellowish

Sl. No	Parameters	Observation
3	Taste	Bitter
4	Oduor	Oduorless

b. Solubility

- Solubility studies of pure drug in different solvents:

Baclofen is freely soluble in Acetone, Chloroform, Methanol and Ethanol and results are shown below,

Table 4: Solubility study of Baclofen in different Solvents.

Drug	Solubility in acetone mg/ ml	Solubility in Ethanol mg/ml	Solubility in Methanol mg/ml	Solubility in Chloroform mg/ml
Baclofen	16.494	13.216	23.288	18.518

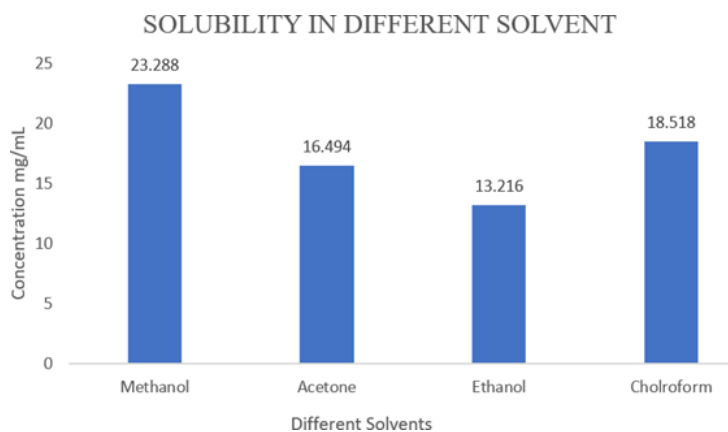


Figure No. 4: Solubility study of Baclofen in different solvents

- Solubility studies of pure drug in different media:

Baclofen shows slight solubility in Phosphate buffer solution (pH 6.8 and 7.4) and distilled water as shown below, Table No 5: Solubility of Baclofen in different media

Drug	Solubility in distilled water (mg/ml)	Solubility in Phosphate buffer (pH 6.8) (mg/ml)	Solubility in Phosphate buffer (pH 7.4) (mg/ml)
Baclofen	0.0049	0.0241	0.0398

SOLUBILITY IN DIFFERENT MEDIA

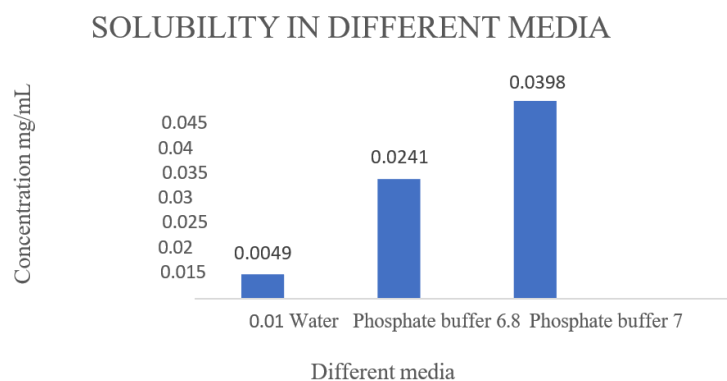


Figure No. 5: Solubility study of Baclofen in different media

Melting point determination

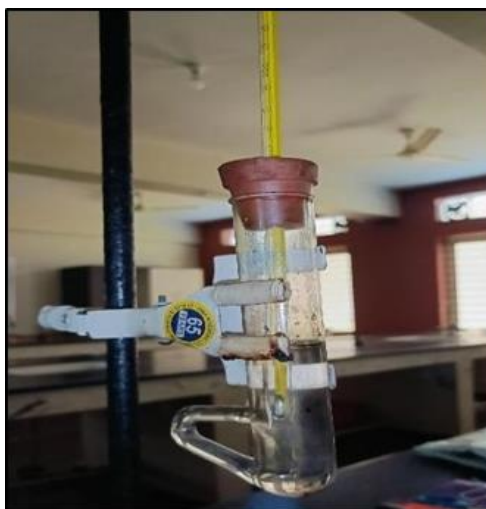


Figure No. 6: Melting point of Baclofen

The melting point of Baclofen is done by Thiele's tube instrument and the melting point is found to be $209^{\circ}\text{C} \pm 0.577$, which was within Pharmacopeial limit of **209- 210°C**

c. Analytical method used in the determination of Baclofen

Determination of λ_{max} of drug

The λ_{max} of Baclofen was obtained from UV-visible spectrophotometer at wavelength ranging from 200-400 nm. The maximum at 223

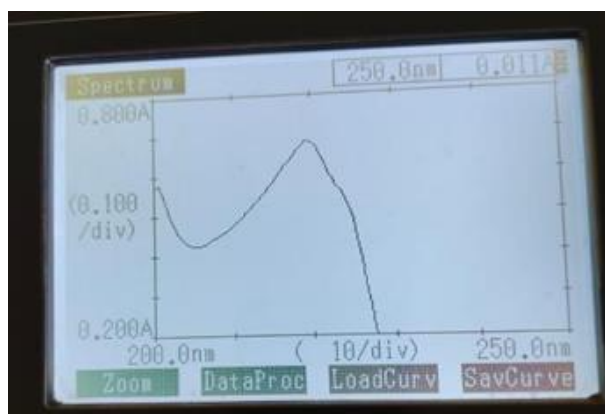
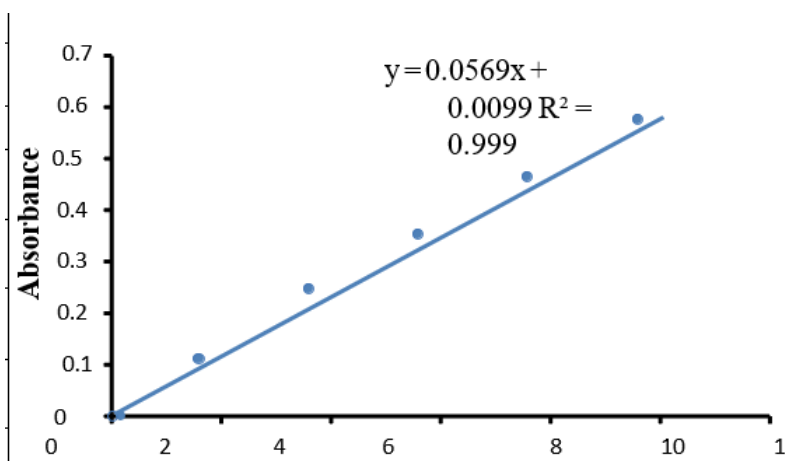


Figure No. 7: Determination of λ_{max} of drug

Calibration curve of Baclofen:

Table No. 06: Calibration curve of Baclofen using phosphate buffer pH 7.4 (N=5)

Sl. No.	Baclofen concentration($\mu\text{g/ml}$)	Absorbance at 220 nm	
		$\bar{X}$	RSD (%)
1	0	0	0
2	2	0.139	0.036 ± 0.139
3	4	0.245	0.021 ± 0.245
4	6	0.351	0.013 ± 0.351
5	8	0.463	0.0016 ± 0.463
6	10	0.575	0.0823 ± 0.575



Calibration curve of Baclofen in Phosphate buffer pH 7.4

1. Compatibility studies of Baclofen with polymers

a. Fourier Transform infrared Spectroscopy (FTIR):

FTIR spectroscopy done by utilizing Bruker alpha, Germany, for Drug, Polymer, Physical and OPT-F.

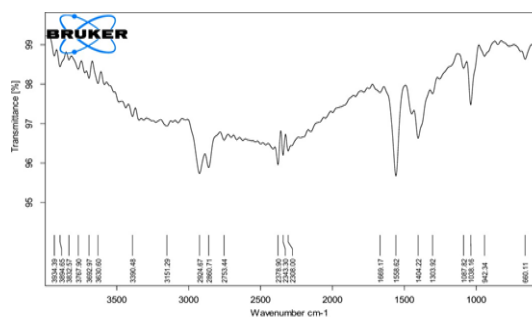


Figure No. 8: IR spectra of Baclofen

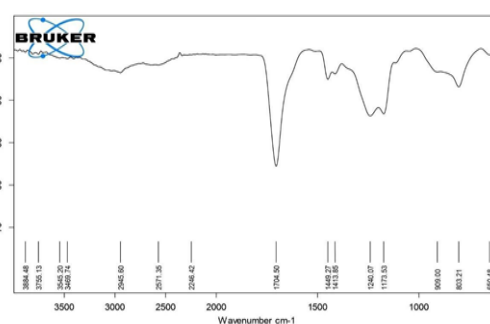


Figure No. 9: PRE-MIX OPT-F.

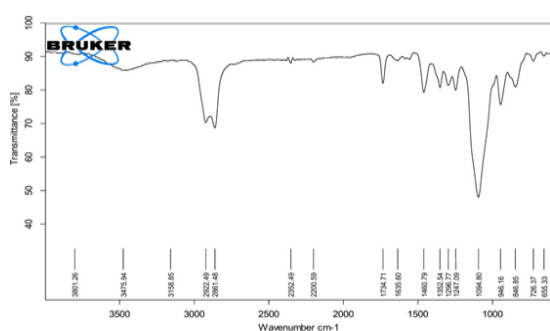


Figure No. 10: IR spectra of sodium deoxycholate

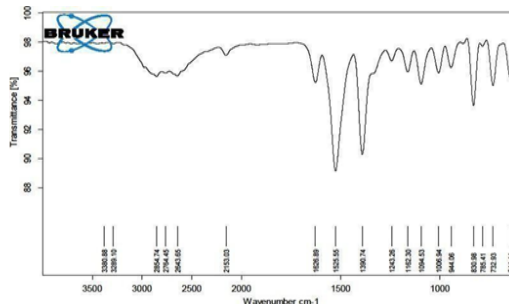


Figure No 11: IR spectra of carbopol 940

Table No 6: Major peaks of Luliconazole and excipients in IR spectra

Functional groups	C-Cl stretch [cm-1]	C-H stretch [cm-1]	C=C-C stretch [cm-1]	C=C stretch [cm-1]	O-H stretch [cm-1]	C≡N stretch [cm1]	C=N stretch [cm-1]
Baclofen	757	2895	1468	1555	2982	2358	1586
Sodium deoxycholate	-	2860	1404	1558	2924	2342	1558
Carbopol 940	-	2945	1413	1449	2945	-	-
PRE-MIX	726	2861	1460	1460	2922	2352	-

DSC Studies

DSC done by utilizing DSC - 60, Shimadzu, for Luliconazole and LBS gel.

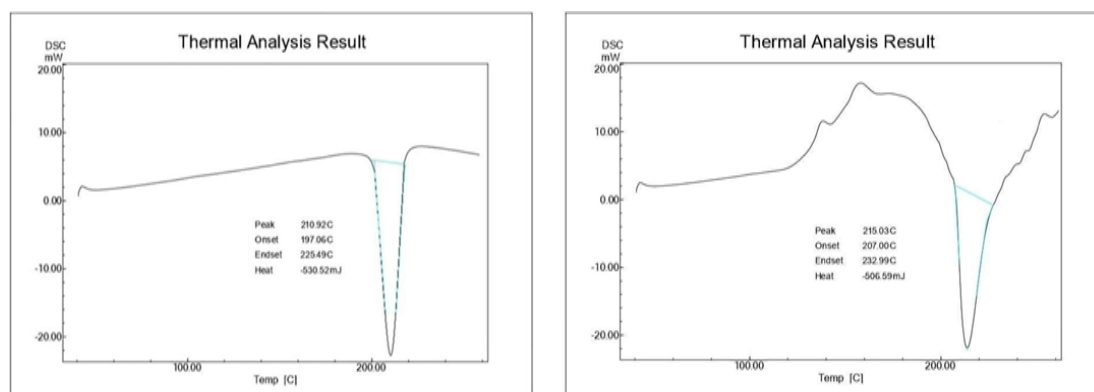


Figure No 12: DSC thermogram of Baclofen and Gel formulation.

Sl. No	Formulation code	Characteristic peak (°C)
1.	Baclofen	210.92
2.	(Optimized formulation)	215.03

Table No. 13: Preparation of Baclofen loaded Bilosomes were prepared by thin film hydration using 2^3 factorial designs.

Formulation Code	X1 Lipids (mg)	X2 SDC (mg)	X3 Span 60 (mg)	X1 Lipids (mg)	X2 SDC (mg)	X3 Span 60 (mg)
	Levels			Actual amount		
F1	0	0	0	35	15	180
F2	+1	+1	-1	40	20	120
F3	0	0	0	35	15	180
F4	0	0	0	35	15	180
F5	-1	+1	-1	30	20	120
F6	-1	-1	+1	30	10	240
F7	-1	-1	-1	30	10	120
F8	+1	-1	-1	40	10	120
F9	+1	+1	+1	40	20	240
F10	+1	-1	+1	40	10	240
F11	-1	+1	+1	30	20	240

50 mg of Pure drug Baclofen and 10 ml of organic solvent (chloroform: methanol) 1:1 kept constant



Figure No.13 Prepared Baclofen loaded Bilosomes

Table No 14: Experimental runs projected, their observed and predicated responses

Runs	Formulation codes	Factor 1 X1: Amount of lipid (mg)	Factor 2 X2: Amount of bile salt(mg)	Factor 3 X3: Amount of Span 60(mg)	Response 1: Y1: Particle size (nm)		Response 2: Y2: Entrapment efficiency (%)	
					Observed values	Predicated values	Observed values	Predicated values
1.	LBS1	35	15	180	191.41	192.4	72.62	72.11
2.	LBS2	40	20	120	171.37	171.56	65.82	65.7
3.	LBS3	35	15	180	192.11	192.4	72.79	72.11
4.	LBS4	35	15	180	191.82	192.42	72.74	72.11
5.	LBS5	30	20	120	165.34	164.72	64.81	64.84
6.	LBS6	30	10	240	219.53	218.95	75.91	75.65
7.	LBS7	30	10	120	161.27	161.47	67.13	67.37
8.	LBS8	40	10	120	163.73	163.11	69.82	69.82
9.	LBS9	40	20	240	221.94	221.34	81.46	80.94
10	LBS10	40	10	240	218.39	217.94	85.06	85.41
11.	LBS11	30	20	240	216.34	217.5	71.52	71.52

ANOVA- Analysis of variance

Table No. 15: Summary of ANOVA applied to 23 factorial designs for Particle size (Y1) Response

Source	Sum of Squares	F-value	p-value	
Model	5821.71	1946.10	< 0.0001	Significant
A-Lipids	20.80	41.72	0.0030	
B-Bile salt	18.30	36.71	0.0037	
C- Span 60	5751.28	11535.33	< 0.0001	
Residual	1.99			
Lack of Fit	1.75	7.09	0.1237	Not significant

Table No. 16: Summary of ANOVA applied to 23 factorial designs for Entrapment efficiency (Y2) Response

Source	Sum of Squares	F-value	p-value	
Model	390.27	9945.02	< 0.0001	Significant
A-Lipids	64.98	11591.03	< 0.0001	
B-Bile salt	25.63	4572.34	< 0.0001	
C- Span 60	268.89	47963.81	< 0.0001	
Residual	0.0168			
Lack of Fit	0.0016	0.2033	0.6963	Not significant

Model Summary Statistics:**Table No 17: Model summary statistics: Influence of formulation variables on the response factors**

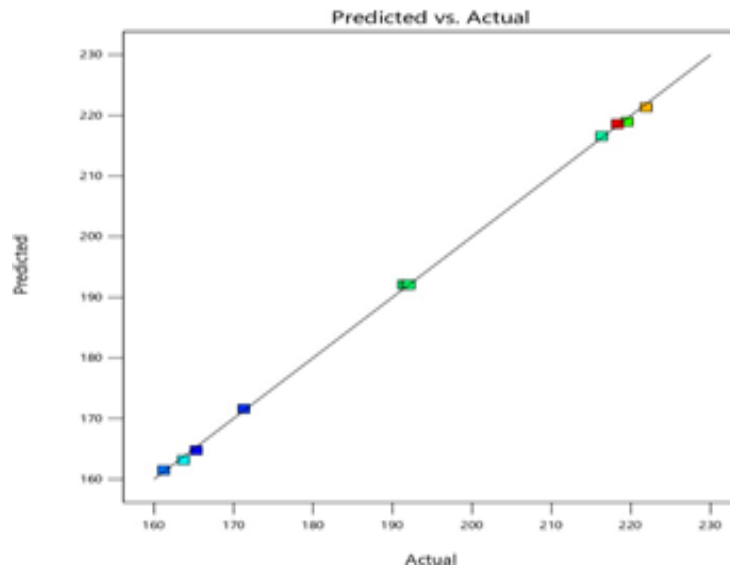
Response factors	Std. Dev	R2	Adjusted R2	Predicted R2	Adequate precision
Y1 Particle size	0.7061	0.9997	0.9991	0.9899	106.298
Y2 Entrapment efficiency	0.0749	1.0000	0.9999	0.9990	317.294

Verification of Model adequacy

The adequacy of the regression model was also verified between the predicted versus actual (experimental data) plots for Y1, Y2 as shown below.

Verification of Model adequacy

The adequacy of the regression model was also verified between the predicted versus actual (experimental data) plots for Y1, Y2 as shown below.

**Figure No. 14: Distribution of actual (experimental) v/s predicted values of the regression model of Particle size (Y1)**

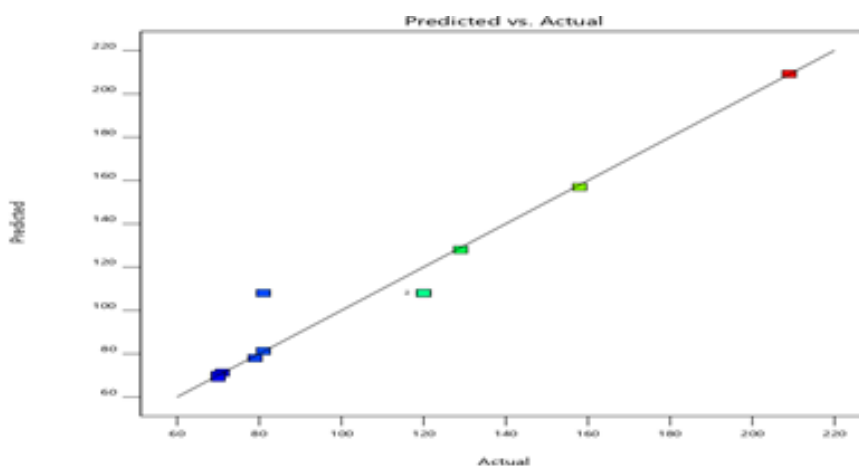


Figure No. 15: Distribution of actual (experimental) vs predicted values of the regression model of Entrapment efficiency (Y2)

Formulation optimization:

Table No. 18: Comparison of predicted and observed response for the statistically optimized formulation

Formulation Code	Response	Observed Value	Predicated Value	Desirability
LBS OPT	Y1: Particle size (nm)	206.21	204.93	1.000
	Y2: Entrapment efficiency (%)	81.17	80.25	1.000

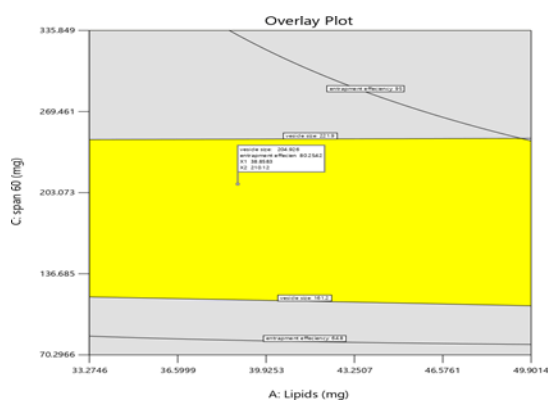


Figure No. 16: Overlay plot of optimized formulation

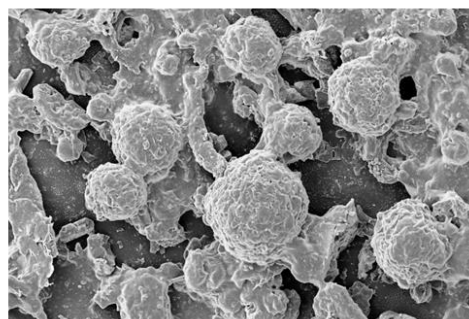
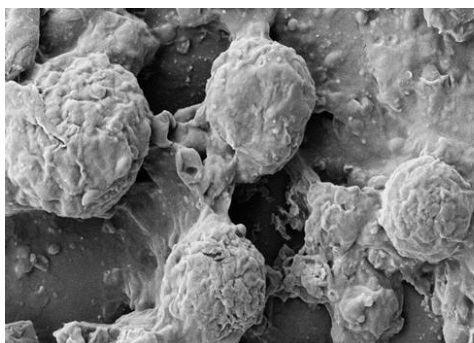


Figure No 17 SEM image of optimized Formulation

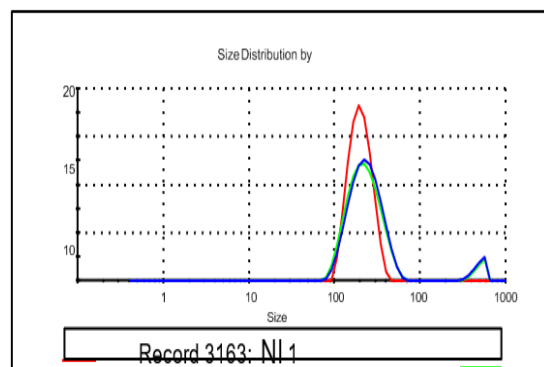
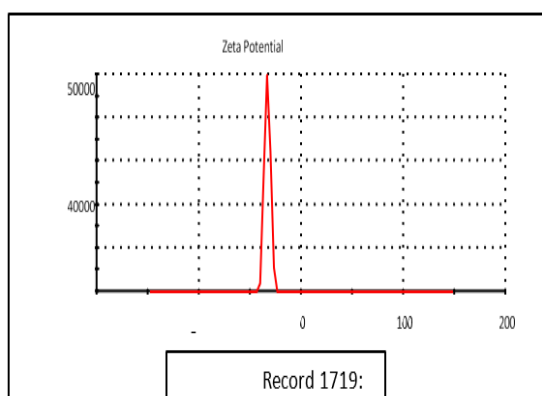


Figure No 36: Zeta potential distribution of OPT-BS.

Table No. 19: Particle size, Poly dispersibility index and Zeta potential of OPT-BS.

Formulation Code	Vesicle Size (nm)	Poly dispersibility index	Zeta Potential (mV)
OPT-BL	200.8	0.301	-31.3

1. Preparation of Baclofen Bilosomal gel by optimized Baclofen loaded Bilosomes.

Bilosomal gel was prepared by optimized Baclofen loaded Bilosomes BBS-OPT.

Formulation table of Baclofen loaded Bilosomes Gel.

Table No. 20: Formulation table of Baclofen loaded Bilosomes Gel

SL NO	Ingredients	Quantity
1.	Carbopol-940 (g)	1 g
2.	Optimized Formulation (ml) OPT	10 ml
3.	Triethanolamine (ml)	0.5 ml
4.	Water (ml)	QS to 100 ml
5.	Methyl and Propyl paraben	0.1%

2. Evaluation of gel containing Baclofen loaded Bilosomes.

a. Appearance and Homogeneity:

Table No. 21: Gel containing Baclofen loaded Bilosomes for Appearance and Homogeneity

Formulation Code	Appearance	Homogeneity
LBS GEL	Off- white Colour	Homogeneous

pH, Spreadability, Extrudability and Viscosity:

Table No. 22: pH, Spreadability, Extrudability and Viscosity of gel containing Baclofen loaded Bilosomes

Formulation Code	pH $\bar{X} \pm \text{RSD} (\%)$	Viscosity (cps) $\bar{X} \pm \text{RSD} (\%)$	Spreadability (mm) $\bar{X} \pm \text{RSD} (\%)$	Extrudability
BLS GEL	5.9 ± 0.04	3760 ± 20.06	59 ± 0.42	Good

Short-term stability studies for prepared gel containing **Baclofen** loaded Bilosomes as per ICH guidelines.

Table No. 22: Evaluation of LBS GEL formulation after stability studies (40±2°C)

SL NO	Evaluation Parameters	Before stability testing	After stability testing
1.	pH	5.9±0.04	5.9±0.36
2.	Viscosity (cps)	3760±20.06	3761±24.33
3.	Spreadability (mm)	59±0.42	59±0.34
4.	Extrudability	Good	Good

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