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"DEVELOPMENT AND EVALUATION OF BILOSOMAL GEL LOADED WITH NON-STEROIDAL ANTI-INFLAMMATORY DRUG"

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ABSTRACT

The study focused on developing and optimizing a Diclofenac sodium bilosomal gel for enhanced topical delivery and sustained anti-inflammatory action. Bilosomes were prepared using the thin film hydration method using soya lecithin, cholesterol, Span 60, and bile salts, Sodium deoxycholate [SDC] / Sodium Tauroglycocholate [STGC] were tried using 3^2 Factorial design and optimized using Design Expert Software (version 13.5). Among 18 [9+9] formulations, SDC-based bilosomes showed superior results, with spherical vesicles (138–310 nm), high entrapment efficiency (84–90%), and sustained drug release up to 12 hours following zero-order kinetics and non-Fickian diffusion. The optimized formulation incorporated into a Carbopol-934 gel demonstrated acceptable pH, viscosity, and drug content, releasing 80.63% of the drug over 12 hours. Ex vivo studies confirmed better skin permeability for bilosomal formulation compared to Diclofenac sodium bilosomal gel and marketed products, indicating that bilosomal gel offers a promising system for effective and sustained topical delivery of Diclofenac sodium.

KEYWORDS: Bilosomes; Diclofenac sodium; Sodium deoxycholate (SDC); Sodium tauroglycocholate; Skin permeation; Carbopol-934 gel; In vitro drug release; Ex-vivo studies.

INTRODUCTION

Particulate delivery methods have attracted a lot of scientific attention in recent decades. In order to alter and enhance the pharmacokinetic and pharmacodynamic characteristics of different kinds of pharmacological molecules, particulate systems such as nanoparticles have been used as physical techniques.[1]

Niosomes and liposomes, in particular, have demonstrated the capacity to entrap hydrophilic and lipophilic medicines. However, the main issues with conventional nano-vesicular carriers were instability in storage and drug leakage.[2,3]

Bilosomes are bile salt-stabilized lipid nanovesicles that offer greater elasticity, flexibility, and membrane deformability than conventional vesicular systems like liposomes and niosomes. Formulated from lipids, surfactants, and bile salts, they can effectively penetrate biological barriers. Due to their structural compatibility, bile salts can be incorporated into traditional vesicles to convert them into bilosomes.[4,5]

Bile salts increase vesicle deformability, which makes it easier for them to pass through skin pores and act as edge activators. Additionally, when applied in non-occlusive environments, their enhanced vesicular flexibility enables them to retain and bind water, facilitating integration with the lipid layers of the skin and ensuring proper hydration.[6]

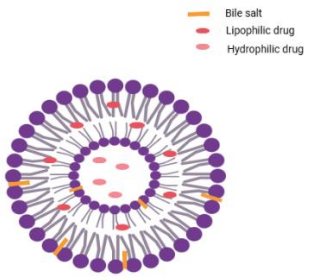

Figure 1: Structure of Bilosomes.

For successful transdermal administration, the bilosomes particle size must be in the nanorange. Sodium deoxycholate and other negative-charged bile salts are used to increase the vesicular system's stability. Transdermal administration is enhanced by its fluidizing properties. Bilosomal systems have demonstrated enhanced stratum corneum (SC) penetration, meeting a crucial prerequisite for effective transdermal drug delivery.[7]

Diclofenac sodium is a nonsteroidal anti-inflammatory drug (NSAID) that belongs to BCS Class II with half life of two hours. Diclofenac Sodium is one of the leading pain relief drugs which acts by COX- enzyme inhibition resulting in anti-inflammatory action. Disadvantages like Gastrointestinal upset, dosing frequency of more than once a day can be overcome by the development of drug delivery system that enables the controlled release of NSAIDs could be highly beneficial particularly in high dose-dependent treatment as in the treatment of chronic diseases such as Rheumatoid arthritis.

The development of a transdermal gels of non-steroidal anti-inflammatory drugs (NSAIDs) loaded in bilosomal carriers is a promising advancement in drug delivery systems. Transdermal gels deliver medication directly to the affected area, ensuring targeted and rapid relief by improving skin penetration. Hence, in the present investigation an attempt was made to formulate transdermal gel of Bilosome containing Diclofenac sodium in which bile salts are included into the carrier to enhance the permeation of therapeutically active drug.

MATERIALS AND METHODS

METHOD OF PREPAPARATION OF BILOSOMES

PREPARATION OF DICLOFENAC SODIUM LOADED BILOSOMES BY THIN FILM HYDRATION METHOD

Lipid thin film hydration method was used in this study for preparation of bilosomes, in this method, 0.5-litre round bottom flask was taken; Diclofenac sodium was dissolved in 5ml methanol. In separate beaker Lecithin, Cholesterol & Span 60 were co-dissolved in a 10ml chloroform. Both organic solutions were mixed and added to Round bottom flask, the organic solvent was evaporated for 2 hour until complete dry film was obtained under reduced pressure using a rotary evaporator. Then this dry film was hydrated 2 hours using 30ml phosphate buffer of pH 7.4 containing Bile salt [SDC or STGC]. Finally, the prepared Diclofenac sodium dispersion was further ultrasonicated for 20 min to reduce the size.[8,9]

Design of experiments

The Design of Expert (DOE) software version 13.0.5 was used for statistical study. Optimization by DOE was done to find out the values of controlled independent variables, that produce the most desirable value of dependent variables. A Central composite Design(CCD) was employed, which involved two quantitative variables- the concentration of Span 60 and concentration of Bile salt is selected as a independent variables. Type of bile salt is selected as a qualitative or categoric factor. In addition to these variables. The amount of Drug, Soyalecithin & Cholesterol were kept constant in all the trials.

The levels of two quantitative variables were selected based on the preliminary trials which were done before using DOE software. The independent variables and their levels were selected based on the evaluations of preliminary trials. The selected dependent variables were particle size, Entrapment Efficiency, % Drug release at 6^{th} hour. $3^2 + 3^2$ factorial design was planned for each type of bile salt. First 9 formulations using one bile salt i.e., Sodium deoxycholate [D1-D9] were carried out individually. Similar design was created for the other type Sodium tauroglycocholate and nine more formulations [T1-T9] were prepared using the same procedure.[10]

Table 1: Test Factors & their levels for the formulation of Diclofenac sodium bilosomes.

Factors (Quantitative factor)	Name	Units	Low Level(-)	Middle Level(0)	High Level(+)
A	Span 60	mg	90	225	360
B	SDC	mg	8	22	36

Table 2: Categoric factor.

Factor	Name	Units	Levels	Level 1	Level 2
Categoric factor **(C)**	Type of bile salt	mg	2	Sodium deoxycholate	Sodium Tauroglycocholate

Table 3: Formulation of Diclofenac sodium Bilosomes using Sodium deoxycholate as a type of bile salt [D1-D9].

Formulation Code	Diclofenac Sodium (mg)	Cholesterol (mg)	Lecithin (mg)	Span 60(mg)	Bile salt (mg)	Type of Bile salt (mg)
D-1	100	36	36	90	8	SDC
D-2	100	36	36	90	22	SDC
D-3	100	36	36	90	36	SDC
D-4	100	36	36	225	8	SDC
D-5	100	36	36	225	22	SDC
D-6	100	36	36	225	36	SDC
D-7	100	36	36	360	8	SDC
D-8	100	36	36	360	22	SDC
D-9	100	36	36	360	36	SDC

Table 4: Formulation of Diclofenac sodium Bilosomes using Sodium deoxycholate as a type of bile salt[T1-T9]

Formulation Code	Diclofenac Sodium(mg)	Cholesterol (mg)	Lecithin (mg)	Span 60(mg)	Bile salt(mg)	Type of Bile salt (mg)
T-1	100	36	36	90	8	STGC
T-2	100	36	36	90	22	STGC
T-3	100	36	36	90	36	STGC
T-4	100	36	36	225	8	STGC
T-5	100	36	36	225	22	STGC
T-6	100	36	36	225	36	STGC
T-7	100	36	36	360	8	STGC
T-8	100	36	36	360	22	STGC
T-9	100	36	36	360	36	STGC

Evaluation of Diclofenac sodium Bilosomes

Particle size

The dynamic light scattering particle size analyzer was used to measure the prepared bilosomes particle size. Prior to measurement and analysis in a Horiba Scientific particle size analyzer, 1 milliliter of the produced formulation was diluted with 10 milliliters of distilled water and sonicated for one minute using a bath sonicator[10] and particle size was measured.

Zeta Potential

Surface charges of all the Bilosomal formulations were determined by zeta sizer. ZP was determined by monitoring the electrophoretic mobility of charged vesicles in an electric field.[11,12] 1 ml of the prepared bilosomal suspension with 10 ml of double-distilled water & placed into the electrode-containing cuvette, which was retained inside the device while the sample's zeta potential was measured.

Drug Content

Diclofenac sodium content in bilosomes was assayed by an UV spectrophotometric method. Bilosomes equivalent to 3mg of drug were dissolved in a mixture of chloroform & methanol & sonicated for 5min After suitable dilution was made with pH 7.4 phosphate buffer solution, absorbance was measured by UV spectrophotometer against blank at λmax 276 nm and drug content was calculated.

%Entrapment efficiency

The EE% of Diclofenac sodium was obtained indirectly by measuring the concentration of unentrapped drug in the dispersion media.[13] Specifically, 1ml of the vesicular suspension was centrifuged at 15000 rpm for 30 min. The supernatent top was separated and diluted with 10ml methanol. 1 ml of solution was further diluted with phosphate buffer 7.4 and measured using a UV-Vis spectrophotometer at 276nm to determine the concentration of unbound drug.

The results were calculated as an average from three measurements, with standard deviation (SD) representing variability. The drug's Entrapment efficiency percentage (EE%) was estimated using the following equation[7]

$$\% EE = \frac{\text{Total drug - unentrapped drug}}{\text{Total drug}} \times 100$$

***In-vitro* Drug Release Study**

The release of Diclofenac sodium from bilosomal formulations was assessed using the Hi- media dialysis membrane-110 bag diffusion technique. Diclofenac sodium bilosomal solution equivalent to 3mg was taken in a dialysis bag which is tied at one end and the other end was also tied after suspension was added and immersed in a beaker containing 200 ml of PBS pH 7.4, which served as the receptor compartment. The receptor media was maintained at 37±0.5˚C and stirred with a

magnetic stirrer at 100 rpm. Aliquots of 3.5 ml sample were collected at regular intervals, and the same volume of medium was replaced each time. The gathered samples were examined with a UV spectrophotometer at 276 nm. The tests were performed in triplicate (n = 3).[7,14]

Study of *in vitro* drug release kinetics

The kinetics of in vitro release of the prepared formulations was assessed using dependent methods (Higuchi, Korsmeyer-Peppas, zero order, and first order models). The data obtained from in-vitro release studies were fitted to the four models mentioned above, and evaluation was performed based on the values of regression coefficients.[15,16]

Criteria for the selection of optimized formulation

[SDC=9]+[STGC=9]=18 formulations were carried out individually using 3^2 factorial design with concentration Span 60 & bile salt as quantitative factors and bile salt as a categoric factor or qualitative factor by keeping all the levels of Span 60 & type of bile salt same across both the sets, 18 formulations were considered under single design.

The formulation optimization was carried out using Design Expert Software with the following parameters: Span 60 (factor A) and bile salt (factor B) were varied within ranges of 90–360 mg and 8–36 mg, respectively, while the type of bile salt (factor C) was set within the range of SDC to STGC. The particle size was selected as a response to be minimized, % Entapment efficiency as a response to be maximised and the drug release at the 6th hour was considered as a response to be maximized to obtain the optimal bilosomal formulation.

Table 5: Recommended optimum formulation by design expert software.

Span 60 (mg)	Bile salt (mg)	Type of Bile salt	Particle size(nm)	% Entrapment Efficiency	% Drug release at 6th hr	Desirability
99.69	8.14	SDC	138.0	89.47	86.03	1.000

The Design of Experiment (DOE) recommended the above formulation containing Span 60 (99.67 mg) and bile salt (8.14 mg) of type SDC where Drug, Soya lecithin & Cholesterol were kept constant. The particle size (138.0 nm), % Entrapment efficiency(89.47%) drug release at the 6th hour (86.03%) were predicted by the DOE, and this optimized formulation was subsequently prepared and evaluated to validate the statistical design.

Surface and shape analysis by using Transmission Electron Microscopy

The surface features of the optimum formulation were identified using a Transmission Electron Microscope. A drop of bilosomal solution was mounted on a clear glass stub for visualization of the bilosomal suspension, air-dried, and coated with gold (Icon, Mumbai) & recorded.

Formulation of Bilosomally Entrapped Diclofenac sodium Gel

An optimized Bilosomal suspension containing Diclofenac sodium equivalent to 1 % w/w was incorporated into gel base composed of Carbopol-934 and purified water.[17]

Carbopol-934 was added to water and soaked overnight in the water to get good dispersion. Methyl paraben and Polyethylene glycol were added. All this are kept on the magnetic stirrer to get good dispersion using a magnetic agitation. This was added with drop by drop Triethanolamine to adjust the pH of the formulation.

Table 6: Formulation of Carbopol-934 gel loaded with Bilosome containing Diclofenac sodium.

SI.No	Ingredients	Gel A	Gel B	Gel C
1	Carbopol-934(mg)	0.25	0.5	0.75
2	Distilled water(ml)	25±5	25±5	25±5
3	Methyl paraben(mg)	0.02	0.02	0.02
4	Bilosome containing Diclofenac sodium (equivalent to)	3	3	3
5	Polyethylene glycol (ml)	5	5	5
6	Gel texture	Low viscous gel	Low viscous gel	Soft gel with good texture

Evaluation of Bilosomal Gel

Physical Appearance: The formulated gel was assessed for transparency, hue, uniformity, and any foreign particles.

pH: The pH of dispersion was measured by using digital pH meter.

Rheological Study by Viscosity Measurement: The viscosity was measured using a Brookfield programmable DV-E viscometer. For this study, spindle number 62 was employed at an ideal speed of 10 rpm to assess the viscosity of the formulation.

Spreadability: The parallel-plate method is the most frequently used technique for assessing spreadability. In this measurement process, 1 g of a sample, which has been prepared 48 hours prior to testing, is positioned between two glass plates. A weight of 100 g is applied to the upper plate for 1 minute. Afterward, the diameter of the sample contained between the plates is measured.

In these cases, spreadability is determined by the formula:

$$Si = d^2 \times \frac{\pi}{4}$$

Where,
Si- spreading area(mm^2) depending on mass,
d- spreading area diameter(mm)

Content Uniformity

Drug content was determined by dissolving 1 g of gel in 10 mL ethanol, followed by filtration through Whatman filter paper No. 41. One ml of the filtrate was diluted to 10 mL with phosphate buffer (pH 7.4). Diclofenac sodium concentration was measured at 276 nm using a Shimadzu UV–Vis spectrophotometer. All measurements were performed in triplicate (n=3).

***In-vitro* Drug Diffusion Study**

The in-vitro diffusion study was performed using a Franz diffusion cell with Hi-Media dialysis membrane-110 pre-soaked in pH 7.4 phosphate buffer. The membrane was placed between the donor and receptor compartments containing 135 mL of pH 7.4 buffer. One gram of bilosomal gel was applied to the donor side, and the assembly was maintained at 37 ± 0.5 °C with continuous stirring, ensuring no air bubbles formed beneath the membrane. At predetermined intervals, 5 mL samples were withdrawn from the receptor compartment and replaced with fresh buffer. Drug content was determined using a UV-visible spectrophotometer at 276 nm. All measurements were conducted in triplicate (n=3).

Ex-Vivo Comparision Studies

Porcine ears, obtained from local slaughter house, were initially washed with cold running water and the outer region of the ear was cut without damaging the Stratum corneum and only epidermal layer was removed carefully using blunt forceps and sharp scissors. The central portion of the external side was excised, dipped in water at 40^0 C to remove subcutaneous fat and cut with a scalpel and molded into a round shape to fit Franz cells. Porcine ear was selected to simulate human skin conditions.

Ex-vivo diffusion study was carried out for Diclofenac Sodium loaded Bilosomes, Diclofenac sodium Loaded bilosomal Gel and MKT product (Diclofenac Gel 1% w/w, Voveran) considering quantity of drug equivalent to 3mg which was placed in intimate contact of pre equilibrated porcine ear mounted Franz diffusion cell, effective surface: 2.1 cm^2, receptor media: 135ml of phosphate buffer pH 7.4, at 37 ± 1°C and 100 rpm. Aliquots of 3.5 ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed using UV spectrophotometer at 276 nm. The tests were carried out in triplicate (n=3). Using the data obtained from the ex vivo study skin parameters like steady state flux(J) was calculated using slope of the linear portion of the cumulative permeation v/s time plot and permeability coefficient(Kp) was determined by measuring the membrane's ability to allow the drug to pass through.

RESULTS AND DISCUSSION

Diclofenac sodium loaded bilosomes were successfully prepared by Thin film Hydration Method.

Table 7: The results of Diclofenac sodium bilosomes using Sodium deoxycholate as a type of bile salt.

Formulation code	Span 60 (mg)	Bile Salt (mg)	Type of Bile salt	Particle size(nm)	% Entrapment Efficiency	%CDR at 6^{th} hr
D-1	90	8	SDC	138.6	85.96	85.33
D-2	90	22	SDC	215	85.19	77.9
D-3	90	36	SDC	238.9	84.54	77.32
D-4	225	8	SDC	156.3	88.01	83.67
D-5	225	22	SDC	151.1	87.96	83.38
D-6	225	36	SDC	275	87.51	71.81
D-7	360	8	SDC	218.5	89.08	77.51
D-8	360	22	SDC	223.3	90.72	77.32
D-9	360	36	SDC	310.9	88.68	65.98

Table 8: The results of Diclofenac sodium bilosomes using Sodium tauroglycocholate as a type of bile salt.

Formulationcode	Span 60 (mg)	Bile Salt (mg)	Type of Bile salt	Particle size(nm)	% Entrapment Efficiency	%CDR at 6th hr
T-1	90	8	STGC	136	92.51	78.1
T-2	90	22	STGC	181.2	89.64	79.08
T-3	90	36	STGC	251.5	86.34	81.32
T-4	225	8	STGC	173.6	91.72	73.9
T-5	225	22	STGC	196.9	88.13	75.65
T-6	225	36	STGC	323.1	85.95	77.81
T-7	360	8	STGC	177.5	84.51	70.67
T-8	360	22	STGC	208.2	90.72	74.87
T-9	360	36	STGC	337.2	88.59	71.65

Response 1: Particle size

Influence of Sodium deoxycholate as Bile salt on Particle size

Linear Equation in Terms of Coded Factors

Particle size = +214.18+26.70*A+51.90*B

A* = Span 60

B* = Sodium deoxycholate

In the above equation both Span 60 & SDC has a positive effect on particle size – as their concentration increases, particle size increase.

The Particle size of all bilosomes prepared using Sodium deoxycholate ranges from 138.60nm to 310.90nm.

The surfactant showed the positive effect on vesicle size. increasing the hydrophilicity of the surfactant led to more water being taken in, which made the vesicles bigger.[10,18] In some cases, increase in surfactant concentration led to reduction in interfacial tension between Cholesterol and aqueous phase and size decreases.[19] This could be attributed to the lower HLB value of Span 60 (4.7). The connection between surfactant HLB and vesicle size can be explained by a reduction in surface energy due to increased hydrophobicity, which leads to the formation of smaller vesicles.[20]

Increasing the amount of SDC from 8mg to 22mg and up to 36mg resulted in a larger size of vesicles. The anionic nature of bile salts and their steroid structure increase Vesicle size in two ways: the first is by growing the internal aqueous core space, and the second is by increasing the steric repulsive force between bilosome bilayers, which may increase bilosome bulkiness.[7,21]

Influence of Sodium tauroglycocholate as Bile salt on Particle size

Linear Equation in Terms of Coded Factors

Particle size = +220.58+25.70*A+70.78*B

A* = Span 60

B* = Sodium tauroglycocholate

According to the above coded equation. both Span 60 & STGC has a positive effect on particle size – as their concentration increases, particle size also increases.

The Particle size of bilosomes all prepared using STGC ranges from 136.0nm to 337.2nm.

Sodium tauroglycocholate also tend to increase the particle size of bilosomes due to its bulkiness, more hydrophilic nature and weaker membrane-disrupting action of the vesicles while SDC has a stronger membrane disrupting action.[22]

Comparision between the effects of SDC & STGC on Particle size as type of bile salt

Type of Bile salt [SDC] = +66.443+0.1914*A+4.782*B

Type of Bile salt [STGC] = +71.732+0.1914*A+4.782*B

The particle size of bilosomes is influenced by Span 60 (A) and bile salt concentration (B), with both factors increasing size. The effect of SDC is stronger than span 60, indicating bile salt plays a major role in controlling vesicle size. STGC formulations show a higher particle size compared to SDC, likely due to its bulkier and more hydrophilic nature. Therefore, SDC is preferred for achieving smaller particles, while STGC may increase vesicle size and drug loading.

Response 2: % Entrapment Efficiency

Influence of Sodium deoxycholate as Bile salt on %Entrapment Efficiency

Linear Equation in Terms of Coded Factors

%EE= +87.52-0.4400*A-2.13*B

A* = Span 60

B* = Sodium deoxycholate

According to the coded equation of entrapment efficiency, Span 60 & SDC showed a negative impact on % Entrapment efficiency.

The %Entrapment Efficiency of bilosomes prepared using SDC ranges from 84.54% to 90.72%.

Span 60 can slightly alter bilayer packing and marginally reduce %EE, whereas higher bile salt concentrations markedly fluidize and disrupt the vesicle membrane, causing drug leakage—hence the larger negative coefficient for Bile salt.[23] In addition, The increased transition temperature, combined with the long alkyl chain of Span 60, may play a role in enhancing the

entrapment of Diclofenac sodium within bilosomes as the concentration of Span 60 increases.[10,24]

SDC has surface active property and is integrated into bilayer membrane surfaces, enhancing lipid membrane flexibility and drug solubility, enhances entrapment efficiency.[25] Further increase of bile salt concentration up to 36mg leads to significant decrease in EE because high bile salt content enhances the possibility of acting as a solubilizing surfactant and lowers vesicle compactness, facilitating drug leakage and decreasing %EE.[26]

Influence of Sodium tauroglycocholate as Bile salt on %Entrapment Efficiency

% Entrapment Efficiency = +88.68-0.7783*A-3.02*B
A* = Span 60
B* = Sodium tauroglycocholate

According to the coded equation of entrapment efficiency, both Span 60 & STGC also showed a negative impact on % Entrapment efficiency. The %Entrapment Efficiency of bilosomes prepared using STGC ranges from 84.51% to 92.51%.

Increasing bile salt concentration from 8 to 22mg entrapment efficiency ranged from 84% to 92%. Sodium tauroglycocholate (STGC) demonstrated greater drug entrapment than SDC. This might be due to its unique chemical structure (mixed bile salt conjugated with both taurine and glycine), higher molecular weight, hydrophilic-lipophilic balance of STGC. At higher concentration of STGC, more of the drug is lost to the external phase because of micelle formation and solubility increases therefore at higher concentration bile salts have negative effect on % entrapment efficiency.[22]

Comparision between SDC & STGC as a type of bile salt on % Entrapment Efficiency

Type of Bile salt [SDC] = +92.58361-0.004512*A-0.184167*B
Type of Bile salt [STGC] = +93.74583-0.004512*A-0.184167*B

The equations show that entrapment efficiency depends mainly on bile salt concentration (negative effect i.e coefficient is -0.184167), with Span 60 playing only a minor role. STGC has a slightly higher baseline EE than SDC, but upon increasing bile salt reduces EE in both cases.

Response 3: % Drug release

Influence of Sodium deoxycholate as Bile salt on *In-vitro* drug release

Drug release at 6^{th} hr = +77.80-3.29*A-5.23*B
A* = Span 60
B* = Sodium deoxycholate

The coded equation indicates that drug release at the 6th hour is primarily influenced by Span 60 (A) and bile salt (B). The positive intercept represents the baseline release, while the negative coefficients show that increasing either factor reduces drug release. Among the two, bile salt has a stronger suppressive effect due to its larger coefficient.

Influence of Sodium tauroglycocholate as Bile salt on in-vitro drug release

Drug release at 6^{th} hr = +75.89-3.55*A+1.35*B
A* = Span 60
B* = Sodium tauroglycocholate

The coded equation represents that Span 60 has negative effect while STGC shows positive effect on drug release.

Comparision between SDC & STGC as type of bile salts on in vitro drug release

Drug release at 6th hr(%)

Type of Bile salt [SDC] = +64.787+0.0170*A+0.3738*B
Type of Bile salt [STGC] = +64.768+0.0170*A+0.3738*B

Span 60 has a small positive effect on release, while bile salt concentration strongly increases it. Both SDC and STGC influence release similarly, with SDC slightly higher. Overall, drug release depends mainly on bile salt concentration rather than Span 60, with minimal difference between the two bile salts.

Mechanism behind Drug release

The in-vitro release of Diclofenac sodium from bilosomal formulations ranged from 65.98% to 85.33%. All formulations exhibited a biphasic pattern with an initial burst during the first 2 hours, followed by sustained release up to 12 hours, reaching nearly 95% release overall. The burst effect is attributed to surface-adsorbed drug, while prolonged release results from drug diffusion through the hydrophobic bilayer. Additionally, bile salts contributed to enhanced release by improving vesicle permeability and drug solubility within the bilosomal matrix.[27,28]

Both Span 60 and SDC positively influenced the cumulative drug release from bilosomes, as higher levels of these components improved vesicle flexibility and facilitated Diclofenac sodium diffusion. The use of Span 60, with its low HLB value (4.7), further promoted easier drug release and permeation by enhancing bilayer fluidity.[27]

DOE results indicated that SDC produced smaller particle sizes and slightly better drug release than STGC. Although both bile salts followed similar trends, the higher baseline release and reduced vesicle size with SDC make it the more suitable option for bilosomal formulations.

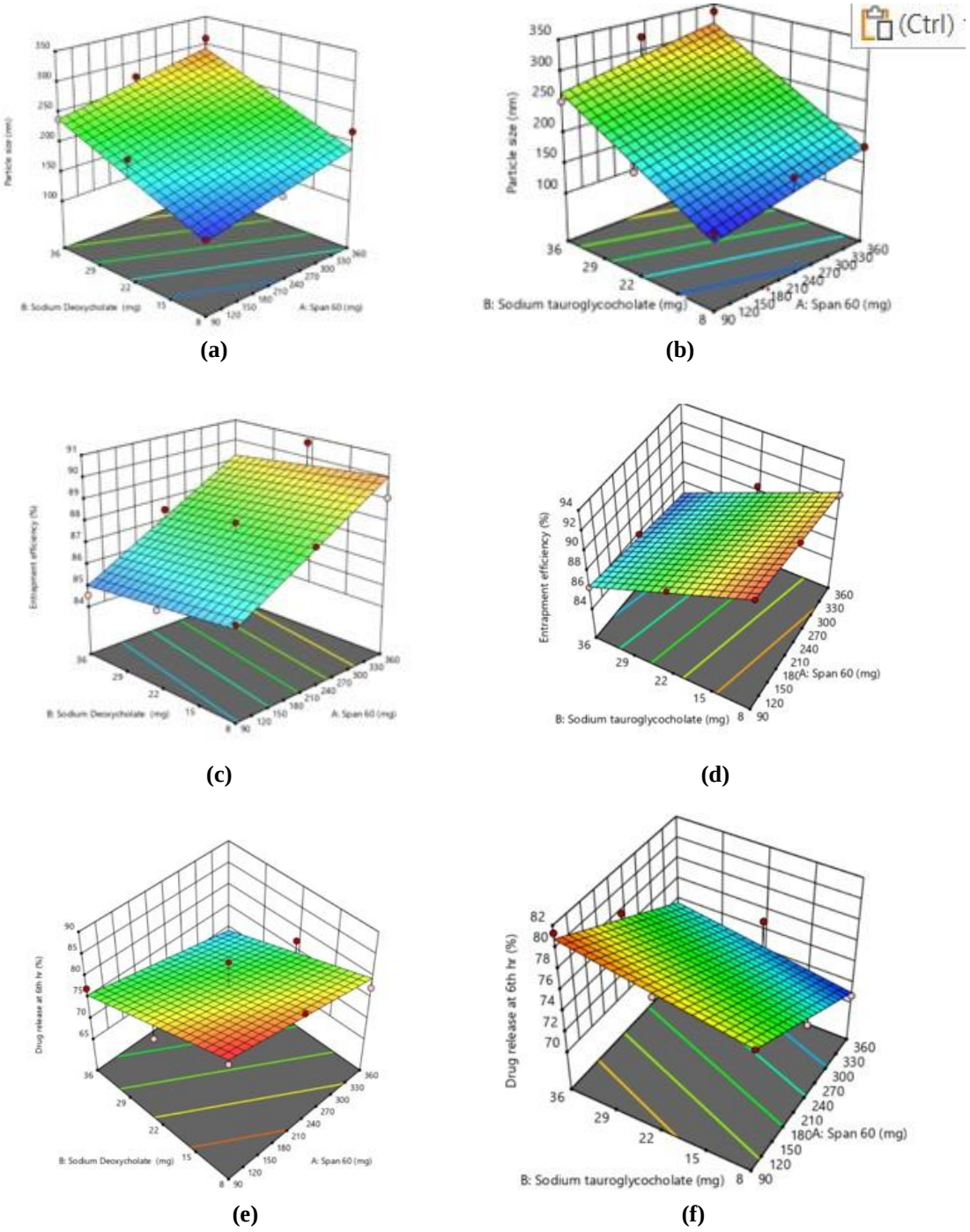

Fig 2: (a), (c) & (e) depicts the 3-D graph of particle size, %Entrapment efficiency & Drug release of Bilsomes using Sodium deoxycholate as a type of bile salt And Fig (b), (d) & (f) depicts 3-D graph of particle size, %Entrapment efficiency & Drug release of Bilsomes using Sodium tauroglycocholate as a type of bile salt.

The evaluation of entrapment efficiency and drug release revealed nearly similar performance for both SDC and STGC. However, particle size showed a clear difference, with SDC producing consistently smaller vesicles. So SDC was selected as the preferred bile salt. Thus, particle size served as the deciding factor in finalizing the best formulation. And in that set of 9 formulations, optimized formulation was selected and further evaluation are carried out.

Numerical optimization

The optimised formulation suggested the composition as follows:

99.674mg of span 60 & 8.14mg of SDC and keeping drug, cholesterol and soyalecithin as constant with a desirability of 1.000. The expected response at those concentration of process variables were 138.0 nm for particle size, 86.03% for *In-vitro* cumulative drug release after 6th hour.

For validating this model, the recommended formulation was prepared and evaluated.

The results are found to be 139.5nm of particle size, and 87.29% *In-vitro* cumulative drug release after 6th hour. The obtained practical results were found to be in close agreement with the model predicted response.

Table 9: Comparision of DOE predictions of optimum formulation evaluation versus actual results.

	Span 60 (mg)	SDC (mg)	Particle size(nm)	%Entrapment efficiency	% Drug release at 6^{th} hr	Desirability
DOE Prediction	**99.697**	**8.141**	**138.017**	**89.47**	**86.037**	**1.000**
Actual results	**99.697**	**8.141**	**139.5**	**87.24**	**87.29**	

Drug Release Kinetic Data Analysis

Table 10: *In-vitro* drug release parameters of optimized formulation.

Optimized formulation	Zero order	First order	Higuchi model	Peppas model		Mechanism of release
	R^2	R^2	R^2	R^2	n value	Non-fickian diffusion
	0.9029	0.8162	0.9856	0.4606	0.9755	

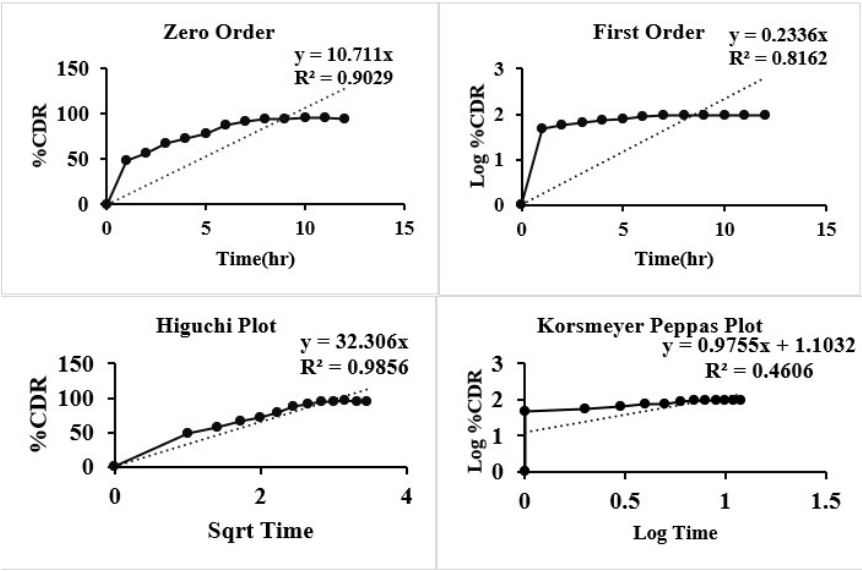

Figure 3: Drug release kinetics of Optimized Diclofenac sodium Bilosomes.

The *in vitro* drug release shown that the regression coefficient values of optimized formulation for Zero order (R^2= 0.9029), Higuchi's model (R^2= 0.9856), Peppas model (R^2= 0.4606), First order (R^2 = 0.8162). The value of regression coefficient (R^2) for Higuchi model is highest. Hence, formulation follow zero order kinetics and Non Fickian diffusion release mechanism.

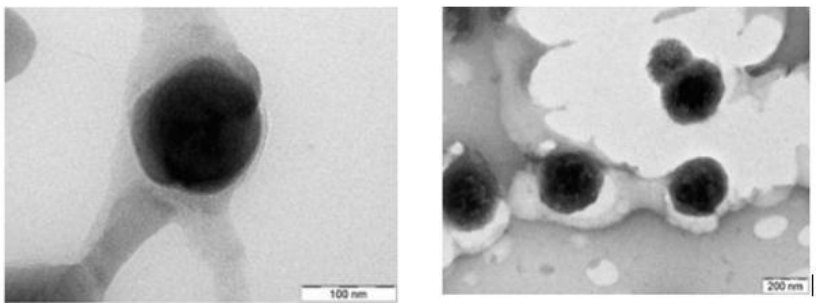

Figure 4: TEM Images of optimized Bilosomes.

Surface analysis and shape by using Transmission Electron Microscopy

TEM analysis of the optimized bilosomal formulation showed uniformly distributed spherical vesicles (130–200 nm) with smooth and intact surfaces, confirming stable lipid bilayer formation. These findings validate the successful development of well-formed bilosomes suitable for efficient drug delivery.

1. **Gel A (0.25%):** This formulation contained the lowest concentration of Carbopol (0.25%), resulting in a low viscous gel. Such a formulation may spread easily but lacks firmness and stability.
2. **Gel B (0.50%):** Increasing the Carbopol concentration to 0.5% slightly enhanced the viscosity, but the gel still remained low viscous. The texture is improved compared to Gel A, but it still may not have sufficient structural integrity for optimal topical application.
3. **Gel C (0.75%):** With 0.75% Carbopol, the gel exhibited soft consistency and good texture, increasing viscosity and forming a smooth, stable, and uniform gel suitable for topical delivery. This concentration appears to achieve an ideal balance between spreadability and firmness.

Evaluation of Bilosomal Gel

Table 11: Results of Various Evaluation Parameters of Diclofenac sodium Bilosomal Gel C.

Parameter	Appearence	Homogienity	pH	Viscosity	Spreadability	% Drug content
Results	Off-white	Good	6.65	2423cp	11.33cm^2	92.25±0.4998

***In-vitro* Drug Diffusion Study**

Table 12: Cumulative Drug Release studies Diclofenac sodium Bilosomal gel.

Time(hr)	1	2	3	4	5	6	7	8	9	10	11	12
%CDR	33.65± 0.0081	40.77± 0.0088	48.16± 0.0088	51.07± 0.0081	54.10± 0.0085	57.00± 0.0091	61.75± 0.0093	64.92± 0.0087	71.92± 0.0081	74.03± 0.0082	78.12± 0.0083	80.63± 0.0081

The diffusion studies focused on the optimized gel, which exhibited drug release percentages ranging from 33.65±0.0081 % to 80.63±0.0081%. Carbopol-934, known for its high viscosity and matrix formation, demonstrated a slow & sustained drug release from the gel.

Ex-vivo comparision studies

Table 13: The study state flux and permeability coefficient.

Formulation	Fss(µg/cm2/hr)	Kp(cm/hr)
Bilosomal gel	28.31	9.436
MKT Product	29.632	9.877
Bilosomes	35.708	11.902

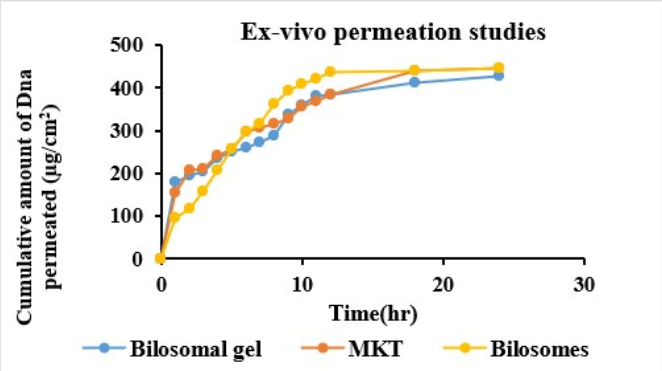

Figure 5: Graph showing Ex vivo comparision study of Bilosomal Gel, MKT product and Bilosomes.

The ex vivo studies demonstrate that **bilosomes** released a higher amount of Diclofenac sodium compared to both the marketed product and the gel formulation. The reasons for enhanced drug release from bilosomes are :

- **Increased Membrane Permeability and Fluidity:** The incorporation of bile salts acts as a fluidizing agent, reducing the stiffness of the lipid bilayer and increasing its hydration and permeability.[29,30]
- **Bile Salt Incorporation:** The presence of bile salts in the bilosome membrane lowers the phase transition temperature, making the vesicles ultra-flexible at physiological temperatures and significantly increasing skin penetration.[31]
- **Enhanced Permeation:** Bile salts themselves have the ability to increase the permeability of drugs across biological barriers, including the skin, by enhancing interaction with the lipidic skin layers.[30,32]

CONCLUSION

Diclofenac sodium loaded bilosomes were successfully formulated using the Thin Film Hydration Method and optimized through a 3^2 full factorial design using Design Expert Software (v13.5). Among 18 formulations containing SDC=9 and STGC=9 as a type of bile salts, the SDC-based formulation exhibited the most desirable particle size. The optimized formulation containing Sodium deoxycholate, producing smooth, spherical vesicles as confirmed by TEM analysis, following zero-order kinetics with a non-Fickian diffusion mechanism. The optimized bilosomes were incorporated into a Carbopol-934 based gel. The bilosomal gel released 80.63% of the drug in 12 hours, and ex vivo permeation studies demonstrated effective and sustained release compared to the marketed formulation.

REFERENCES

1. Prerna, Anubhav Dubey, Ratan Gupta. Nanoparticles: An Overview. Drugs and Cell Therapies in Haematology, 2021 Jun 16; 10(1): 1487–97.
2. Sultan AA, El-Gizawy SA, Osman MA, El Maghraby GM. Niosomes for oral delivery of nateglinide: *in situ–in vivo* correlation. Journal of Liposome Research, 2018 3; 28(3): 209–17.
3. Abd El Azim H, Nafee N, Ramadan A, Khalafallah N. Liposomal buccal mucoadhesive film for improved delivery and permeation of water-soluble vitamins. International Journal of Pharmaceutics., 2015; 488(1–2): 78–85.
4. Elkomy MH, Alruwaili NK, Elmowafy M, Shalaby K, Zafar A, Ahmad N, et al. Surface-Modified Bilosomes Nanogel Bearing a Natural Plant Alkaloid for Safe Management of Rheumatoid Arthritis Inflammation. Pharmaceutics, 2022; 3; 14(3): 563.
5. Nayak D, Rathnanand M, Tippavajhala VK. Unlocking the Potential of Bilosomes and Modified Bilosomes: a Comprehensive Journey into Advanced Drug Delivery Trends. AAPS PharmSciTech., 2023; 21; 24(8): 238.
6. Ammar HO, Mohamed MI, Tadros MI, Fouly AA. Transdermal Delivery of Ondansetron Hydrochloride via Bilosomal Systems: In Vitro, Ex Vivo, and In Vivo Characterization Studies. AAPS PharmSciTech., 2018; 19(5): 2276–87.
7. Ahmed S, Kassem MA, Sayed S. Bilosomes as Promising Nanovesicular Carriers for Improved Transdermal Delivery: Construction, in vitro Optimization, ex vivo Permeation and in vivo Evaluation. IJN., 2020; 15: 9783–98.
8. Khalil RM, Abdelbary A, Kocova El-Arini S, Basha M, El-Hashemy HA. Evaluation of bilosomes as nanocarriers for transdermal delivery of tizanidine hydrochloride: *in vitro* and *ex vivo* optimization. Journal of Liposome Research, 2019; 29(2): 171–82.
9. Albash R, El-Nabarawi MA, Refai H, Abdelbary AA. Tailoring of PEGylated bilosomes for promoting the transdermal delivery of olmesartan medoxomil: in-vitro characterization, ex-vivo permeation and in-vivo assessment. IJN., 2019; 14: 6555–74.
10. Mahmoud TM, Nafady MM, Farouk HO, Mahmoud DM, Ahmed YM, Zaki RM, et al. Novel Bile Salt Stabilized Vesicles-Mediated Effective Topical Delivery of Diclofenac Sodium: A New Therapeutic Approach for Pain and Inflammation. Pharmaceuticals., 2022; 15(9): 1106.
11. Scognamiglio I, De Stefano D, Campani V, Mayol L, Carnuccio R, Fabbrocini G, et al. Nanocarriers for topical administration of resveratrol: A comparative study. International Journal of Pharmaceutics., 2013; 440(2): 179–87.
12. Yen Thi Hai T, Giang Ngoc T, Anh Lan H, Giang Thi Thu V. Niosomes loaded with diclofenac for transdermal administration: Physico-chemical characterization, ex vivo and in vivo skin permeation studies. J Appl Pharm Sci., 2020; 5; 10(12): 053-61.
13. Abdelbary AA, AbouGhaly MHH. Design and optimization of topical methotrexate loaded niosomes for enhanced management of psoriasis: Application of Box–Behnken design, in-vitro evaluation and in-vivo skin deposition study. International Journal of Pharmaceutics., 2015; 485(1–2): 235–43.
14. Khafagy ES, Almutairy B, Abu Lila A. Tailoring of Novel Bile Salt Stabilized Vesicles for Enhanced Transdermal Delivery of Simvastatin: A New Therapeutic Approach against Inflammation. Polymers., 2023; 15(3): 677.
15. Atul Kuksal,1 Ashok K. Tiwary,1 Narendra K. Jain,2 and Subheet Jain. Formulation and In Vitro, In Vivo Evaluation of Extended- release Matrix Tablet of Zidovudine: Influence of Combination of Hydrophilic and Hydrophobic Matrix Formers. AAPS PharmSciTech., 2006; 7(1): 1-9.
16. Paulo Costa, Jose Manuel Sousa Lobo. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences, 2001; 13: 123–33.
17. Satyabrata Bhanja, P.Kishore, Kumar 1, Muvvala Sudhakar1, Arun kumar Das 2. Formulation and Evaluation of Diclofenac transdermal gel. J Adv Pharm Edu & Res., 2013; 3(3): 248–59.
18. Abdelbary GA, Aburahma MH. Oro-dental mucoadhesive proniosomal gel formulation loaded with lornoxicam for management of dental pain. Journal of Liposome Research., 2015; 25(2): 107–21.
19. Zafar A, Alruwaili NK, Imam SS, Hadal Alotaibi N, Alharbi KS, Afzal M, et al. Bioactive Apigenin loaded oral nano bilosomes: Formulation optimization to preclinical assessment. Saudi Pharmaceutical Journal, 2021; 29(3): 269–79.
20. Nagarwal RC, Kant S, Singh PN, Maiti P, Pandit JK. Polymeric nanoparticulate system: A potential approach for ocular drug delivery. Journal of Controlled Release, 2009; 136(1): 2–13.

21. Chettupalli AK, Bukke SPN, Udom GJ, Saraswathi TS, Rahaman SA, Rai SN, et al. Investigating New Bilosomes for Ex vivo Skin Deposition, In Vitro Characterization, and Transdermal Delivery of Nimodipine. Nanoscale Adv., 2024; 10: 1039.
22. Ibrahim TM. Exploitation of transdermal nanobilosomal gel platforms for ameliorating anti-diabetic activity of empagliflozin following I-optimal design. Journal of Drug Delivery Science and Technology, 2023; 84: 104455.
23. Almutairy BK, Khafagy ES, Aldawsari MF, Alshetaili A, Alotaibi HF, Abu Lila AS. Tailoring of Bilosomal Nanogel for Augmenting the Off-Label Use of Sildenafil Citrate in Pediatric Pulmonary Hypertension. ACS Omega, 2024; 9(17): 19536–47.
24. Bnyan, R, Khan, I, Ehtezazi, T, Saleem, I, Gordon, S, Neill, FO and Roberts, M. Surfactant effects on lipid-based vesicles properties: a review. Jornal of Pharmaceutical Science, 2018; 107: 1237−1246.
25. Naji GH, Al Gawhari FJ. Evaluation of types and concentration of bile salts impact on physical properties of nisoldipine-loaded bilosomes. PHAR, 2024; 71: 1–7.
26. Heba A. Ghanem a, Nashwa H. Abd Elwahaba, Mamdouh Ghorab b, Ali M. Nasr c,d, Shadeed Gad b. Bilosomes as a Versatile Drug Delivery System: Preparation Techniques and Biomedical Application. Records of pharmaceutical and biomedical sciences, 2024; 3(8): 67–86.
27. Saifi Z, Rizwanullah Md, Mir SR, Amin S. Bilosomes nanocarriers for improved oral bioavailability of acyclovir: A complete characterization through in vitro, ex-vivo and in vivo assessment. Journal of Drug Delivery Science and Technology, 2020; 57: 101634.
28. Nabil K Alruwaili, Syed Sarim Imam, Mohd Yasir, Omar Awad Alsaidan, Ali Alquraini ,Alenazy Rawaf, Bader Alsuwayt 6,Md. Khalid Anwer 7ORCID,Sultan Alshehri 2ORCID andMohammed M. Ghoneim by AZ. Development and Optimization of Nanolipid-Based Formulation of Diclofenac Sodium: In Vitro Characterization and Preclinical Evaluation, 2022; 14.
29. Said AR, Arafa MF, El-Dakroury WA, Alshehri S, El Maghraby GM. Bilosomes and Niosomes for Enhanced Intestinal Absorption and In Vivo Efficacy of Cytarabine in Treatment of Acute Myeloid Leukemia. Pharmaceuticals, 2024; 17(12): 1572.
30. Kaurav H, Tripathi M, Kaur SD, Bansal A, Kapoor DN, Sheth S. Emerging Trends in Bilosomes as Therapeutic Drug Delivery Systems. Pharmaceutics, 2024; 16(6): 697.
31. Peddapalli H, Radha GV, Chinnaiyan SK. Formulation optimization and PK/PD evaluation of novel valsartan bilosomes enhancing transdermal drug delivery. Journal of Drug Delivery Science and Technology, 2024; 92: 105400.
32. Imam SS, Gilani SJ, Zafar A, Jumah MNB, Alshehri S. Formulation of Miconazole-Loaded Chitosan–Carbopol Vesicular Gel: Optimization to In Vitro Characterization, Irritation, and Antifungal Assessment. Pharmaceutics, 2023; 15(2): 581.