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RESEARCH ARTICLE

DESIGN, FORMULATION, AND *IN-VITRO* CHARACTERIZATION OF LIPID-BASED NANO-BILOSOMAL VESICLES OF LOVASTATIN

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Abstract

Objective: This research work aims to develop the bilosomal vesicles for the delivery of lovastatin (LVS), a lipid-lowering agent known for its poor aqueous solubility and low absorption, which presents a major challenge in drug delivery and development. This study examinesthe potential of Bilosomes as an innovative vesicular drug delivery system to overcome these issues and limitations with LVS and enhance its therapeutic effectiveness.
Methods: Preliminary studies were conducted to determine the suitable lipid, non-ionic surfactant, and bile salt components and their levels for bilosomal system development. Fifteen formulae were obtained by adopting a Box-Behnken surface design using Design Expert software, prepared using the thin film hydration technique, and characterized in terms of entrapment efficiency (EE%), vesicle size (VS), zeta potential (Zp), and cumulative *in-vitro* release % after 72 hours. The developed LVS-loaded bilosomal formulations were then optimized through the analysis of the characterization results to predict the optimized formula.
Results: The maximum wavelength of LVS was determined at 238 nm after UV scanning, and the calibration curve constructed for LVS in dissolution medium showed a strong linear relationship between absorbance and concentration over the range of 2.5 to 20 µg/ml. The saturation solubility of LVS in Sorenson's phosphate buffer (pH 7.4) containing 1% Sodium Lauryl Sulphate (SLS) was significantly enhanced (2.3 mg/ml) compared to its intrinsic solubility in pure water (0.0013 mg/ml), confirming that it was the best dissolution medium for the study. All Box-Behnken developed LVS-loaded bilosomal formulae exhibited high entrapment efficiencies (EE%), nano-size vesicles with polydispersity index (PDI) values, ranging from 0.218±0.006 to 0.495±0.028 indicating uniform size distribution, negative zeta potential (ZP) values ranging mV suggesting good stability, and cumulative release profile ranging from 19.89±0.049% to 43.27±0.024 % revealing sustained release patterns.
Conclusions: This research paper employed Sorenson's phosphate buffer (pH 7.4) containing 1% SLS as a good dissolution medium for LVS in which it showed greater solubility, and highlights the potentials of LVS-loaded Bilosomes with high EE%, vesicular nano-size, negative zeta potential values, and sustained release patterns as efficient drug delivery system for enhancing the solubility and stability of poorly water-soluble drugs such as LVS.
Keywords: Solubility, Box-Behnken design, bilosomes, thin-film hydration technique, characterization.

INTRODUCTION

Statins are a class of oral lipid-lowering drugs used to reduce cholesterol biosynthesis in the liver through their reversible inhibitory action on the mevalonic acid pathway; they prevent 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) conversion by inhibiting the HMG-CoA reductase enzyme. Consequently, they are mainly used for the treatment of hypercholesterolemia and dyslipidemia and can be prescribed for the prevention of many related serious cardiovascular events[1].

Lovastatin (LVS), is a lipophilic white crystal powder with 404.54 Da molecular weight and175.4 °C melting point. Under normal conditions, it has low aqueous solubility (more than 15 liters of water are required to dissolve 20 mg of lovastatin), but good solubility in organic solvents such as chloroform, methanol, and acetone[2,3]. Delivered as an inactive lactone prodrug, LVS needs to be chemically or enzymatically

converted to the active dihydroxy open acid form in order to elicit its exceptionally potent competitive inhibition of HMG CoA reductase. This is precisely what occurs when it undergoes extensive first pass breakdown in the liver, the primary target organ[4,5].

Bilosomes are nano-sized elastic bile salt-containing colloidal transporters with a structure similar to niosomes, composed primarily of non-ionic surfactants and lipids, but also containing bile salt[6-8].

Bilosomes have two layers, the innermost layer (aqueous core) which entraps the hydrophilic drugs or antigens while the outermost layer (lipid bilayer coated and implanted with bile salts) can entrap the hydrophobic dugs. The bile salts are assembled in lipid layers within the bilosomal structure, giving it its characteristic closed form. The hydrophilic end of bile moleculesorients towardthe lipid bilayerhydrophilic region, while the hydrophobic end is immersed in the hydrophobic section of the lipid bilayer in a bilosome vesicle[7,8].

Bile salts are naturally occurring biosurfactants present in the lumen of gastrointestinal tract and are required for lipid degradationand absorption. The most predominantbile salts used in bilosomesdevelopment are sodium glycocholate (SGC), sodium deoxycholate (SDC), and sodium taurocholate (STC). These salts have outstanding solubility qualities, especially for lipophilic molecules, and can successfully encapsulate hydrophobic medicines. They are biocompatible and well-tolerated, making them ideal for drug delivery. They interact with cell membranes, making drugs absorption easier and medicinal constituents more successfully delivered[9].

Nonionic surfactants like Span 40, Span 60, Span 80, Tween 60, and Tween 80 are the surfactant most frequently employed in vesicle preparation due to their greater compatibility, stability, and toxicity compared to anionic, amphoteric, or cationic alternatives. They exhibit reduced irritation, hemolytic activity, and cytotoxicity., serving various functions like wetting agents, emulsifiers, solubilizers, and permeation enhancers[7].

Among several lipids that can be selected for bilosome manufacturing, cholesterol and phospholipids are commonly used due to their amphiphilic properties and their high biocompatibility with biological membranes. Cholesterol is superior due to its beneficial effects on bilosome properties, including increased rigidity, enhanced encapsulation efficiency, improved membrane stability, reduced toxicity, and better rehydration of freeze-dried bilosomes[9].

The efficiency of bilosomes in improving the oral bioavailability of a variety of drugs, sustaining drug release behavior, targeting cancer treatment, and accomplishing various applications such as brain-targeting, herbal, oral vaccination, anticancer, transdermal, and ocular drug delivery has been confirmed by recent research studies[10-15].

The aim of this study was to develop an LVS-loaded bilosomal system as a new oral vesicular carrier for LVS to overcome the problems related to its dissolutions, absorption, and oral bioavailability. A Box-Behnken response surface design was used to statistically analyze the effect of formulation variables.

MATERIALS AND METHODS

Lovastatin (LVS) (purity>98%) was obtained from Sterling Biotech, Ltd, India. Cholesterol was purchased from Alpha Chemika Pvt Ltd India. Sodium deoxycholate (SDC), sodium taurocholate (STC), span 60 (extra pure) and Span 80 (extra pure) were purchased from LobaChemie Pvt Ltd, Mumbai, India. Chloroform (HPLC grade) was purchased from Sigma-Aldrich, Darmstadt, Germany. Nylon syringe filter 0.22 µm was purchased from membrane solutions, LLC, AUBRUN, Washington, USA. Ethyl alcohol (absolute) and anhydrous disodium hydrogen phosphate (sodium phosphate dibasic) were purchased from El-Nasr pharmaceutical chemical co, Egypt. Sodium phosphate monobasic was purchased from Morgan chemical Ind.co, Egypt. Sodium lauryl sulphate (SLS) extra pure powder was purchased from Oxford laboratory reagent, Mumbai, India. Any other ingredients used were of chromatographic or analytical grade and used without further modification or purification.

Quantitative analysis of LVS

Quantitative analysis of LVS was done by validated UV spectrophotometry method. For UV spectro-photometer, 0.1 mg/ml stock solution of LVS in dissolution medium was prepared (10 mg LVS in 100 mL Sorenson's phosphate buffer solution of pH 7.4 containing 1% sodium Lauryl sulphate)[16,17].

Serial dilutions were prepared within a concentration range of (0–20 µg/ml) to construct the calibration curve. Absorbance values for the different sample solutions were recorded spectrophotometrically (HITACHI U-2900 UV/VIS Spectrophotometer, Hitachi High Technologies Corporation, Germany) at λ_{max} 238 nm[18]. By fitting the absorbance into the regression equation obtained from the calibration curve LVS concentration was determined.

Saturation solubility study of LVS

The saturated solubility of LVS in the dissolution medium was determined by adding an excess amount (80 mg) of LVS powder was added to 20 ml dissolution medium (Sorenson's phosphate buffer solution of pH 7.4 containing 1% sodium Lauryl sulphate) in conical flask and covered tightly, then firmly fixed in water bath shaker (Clifton Shaking bath, NE5-28D, NICKEL-ELECTRO LTD, England)[19]. The shaking was done for 48 hours at a speed of 100 rpm and the temperature was maintained at around 37±0.5 °C. At a predetermined intervals aliquots samples were withdrawn and filtered using 0.22 micro-syringe filter to remove the undissolved drug and the LVS absorbance and consequently its concentration was determined spectrophotometrically at λ_{max} 238 nm by time till constant concentration obtained[20].

Preliminary studies and selection of components

The literature was reviewed preliminarily to determine the suitability of different lipid components for drug-loaded bilosome formulation. Based on the available data, cholesterol and phospholipids were assessed and

compared in terms of their ability to enhance the stability, solubility, and bioavailability of poorly water-soluble drugs, guiding the selection of the most appropriate lipid component for the current study. In addition, preliminary experiments shown in Table 1 were performed to evaluate the effectiveness of two surfactants, Span 60 and Span 80, in forming stable bilosomal vesicles. Furthermore, the impact of two bile salts, sodium deoxycholate (SDC) and sodium taurocholate (STC), was investigated to determine their role in improving drug solubility and enhancing the stability of the bilosomal vesicles. These evaluations informed the selection of the optimal surfactant, bile salt, lipid component, and their initial levels for further formulation optimization. The objective was to achieve the desired balance between vesicle stability, size, and drug entrapment efficiency by selecting the optimal surfactant, bile salt, lipid component, and their initial levels.

The variables tested in the preliminary formulations are as follows:

- **Surfactant Types**: Span 60 and Span 80.
- **Surfactant: Lipid Ratio**: High (9:1), Medium (8:2), Low (6:4).
- **Bile Salt Types**: Sodium deoxycholate (SDC) and Sodium taurocholate (STC).
- **Bile Salt Levels**: High (5 mg), Medium (3), Low (1).
- **Lipid Component**: Cholesterol at varying levels (12.5 mg, 10 mg, and 7.5 mg).

Table 1: Preliminary formulations and their component levels investigated for LVS-loaded bilosomal development.

Batch	Surfactant	Lipid (mg)	Bile salt (mg)	Surfactant: Lipid ratio	Bile salt type
F1	Span 60	12.5	5	9 : 1	SDC
F2	Span 80	12.5	5	9 : 1	
F3	Span 60	7.5	1	6 : 4	
F4	Span 80	7.5	1	6 : 4	
F5	Span 60	10	3	8 : 2	
F6	Span 80	10	3	8 : 2	
F 7	Span 60	5	1	6 : 4	
F 8	Span 80	5	1	6 : 4	
F9	Span 60	12.5	5	9 : 1	STC
F10	Span 80	12.5	5	9 : 1	
F11	Span 60	5	1	6 : 4	
F12	Span 80	5	1	6 : 4	
F 13	Span 60	10	3	8 : 2	
F 14	Span 80	10	3	8 : 2	

The amount of LVS is fixed to (20 mg/ 10 ml).

Fabrication of LVS-encapsulated bilosomes

The LVS bilosomal vesicles were prepared by the thin-film hydration technique as described by Chen *et al*, but with slight modifications demonstrated by Hegazy *et al*[21,22]. The amount of Span surfactant and cholesterol along with fixed amount of LVS (20 mg) were dissolved in 250 ml round bottom flask containing 10 ml chloroform/ethanol (7:3) mixture solvent using bath sonicator (Ultrasonic Bath, BRANSON CPX8800H-E, Branson Ultra Sonics Corporation, USA) at 40ºC for (5- 10) min. The 250 mL round-bottom flask containing the dissolved mixture was then attached to the rotary evaporator (Heidolph Rotary evaporator, Basis Hei-VAP HL Adv/pre (EU); Heidolph Instruments GmbH & Co. KG, Germany) operating at120 rpm and 60ºC for 15 min in order to evaporate the organic solvents completely under reduced pressure and obtain a dry thin film of the components, then the dry thin film obtained was hydrated with 10 ml Sorenson's phosphate buffer containing SDC under atmospheric pressure for 1 hour to obtain LVS-loaded bilosomes of milky appearance. Further, the prepared bilosomes were subjected to homogenization (2 cycles of 5 min with gap of 5 min between them at 5000 rpm) through silent crusher homogenizer (Heidolph silent crusher Mhomogenizer, D-91126 Schwabasch Heidolph instrument, Germany), in order to reduce the vesicles size. The attained formulae were then kept at 4 °C for further characterization and to stabilize overnight.

Experimental design for the preparation of LVS-bilosomes

Design Expert® software version 11 (StatEase, Inc., Minneapolis, MN, USA) was used with a Box-Behnken design to analyze the influence of various formulation parameters on LVS-loaded bilosomes[23].

Fifteen runs illustrated in Table 2 were obtained from the constructed design, in which the factors under investigations were set as follow: (X1) Amount of surfactant (Span 80) [90, 52.5, and 15 mg], (X2) Amount of lipid (cholesterol) [10, 7.5, and 5 mg], and (X3) Bile salt (SDC) amount [5, 3, and 1 mg] each at 3 levels as mentioned, resembling the independent variables. Meanwhile, the Entrapment efficiency percentage (EE%) (Y1), Vesicles size (VS) (Y2), Zeta potential (ZP) (Y3) and *in-vitro* drug release percentage (Y4) were selected as the dependent variables[23].

Physicochemical Characterization of the prepared LVS-loaded bilosomes

Entrapment Efficiency Percentage EE (%)

The ability of the developed bilosomal vesicles to enclose LVS was investigated via calculating the EE (%) indirectly through cooling ultracentrifugation procedures as follows[24]: One ml of LVS bilosomal dispersion (resembling 2 mg of the drug) was

transferred by micropipette into 2 ml Eppendorf and then subjected to a cooling centrifugation process at 15,000 rpm and 4ºC for 1 h (HERAEUS BIOFUGE PRIMOR centrifuge, Thermo-Fisher SCIENTIFC, Germany), which in turn allowed the unentrapped or free LVS to separate from the bilosomal pellets that precipitated, then the clear supernatant was carefully withdrawn and filtered through 0.22 micro-syringe filter[25]. The concentration of the unentrapped LVS was determined in the filtered supernatant after appropriate dilution spectrophotometrically at λ_{max} 238 nm, and consequently the EE% was calculated by the following equation:

$$EE\ (\%) = \frac{Td - Fd}{Td}$$

Where; Td= Total amount of drug added, Fd= Free drug estimated in supernatant.

Table 2: The different LVS-bilosomal formulae composition obtained through Box-Behnken design.

Batch	Independent variables		
	X1: Surfactant Amount (mg)	X2: Lipid Amount (mg)	X3: Bile Salt Amount(mg)
F 1	15	10	3
F 2	90	5	3
F 3	15	7.5	1
F 4	52.5	7.5	3
F 5	90	7.5	5
F 6	15	5	3
F 7	52.5	5	5
F 8	52.5	7.5	3
F 9	52.5	10	5
F10	52.5	10	1
F11	15	7.5	5
F12	52.5	5	1
F13	90	10	3
F14	90	7.5	1
F15	52.5	7.5	3

Bilosomal Vesicles Characterization (VS, ZP and PDI)

All bilosomal preparations were sampled (0.5 mL diluted tenfold with distilled water and vortexed for 5 minutes). A sufficient volume of the diluted sample was transferred to a cuvette for measurement of vesicle size, PDI, and zeta potential using the Zeta Sizer Nano ZS (Malvern Instruments, Nano-ZS90, MALVERN Instruments Limited, UK), based on dynamic light scattering (DLS) at 25±2°C. All measurements were performed in triplicate[25].

***In-vitro* release of LVS from different bilosomal formulae**

The release of LVS from different bilosomal formulae was performed using the dialysis bag technique [24]. A dialysis membrane with a 12000–14000 KDA cut-off and 16 mm diameter was used, cut into appropriate segments, and prepared following standard procedures. The membrane was then presoaked for 24 hours in the dissolution medium (Sorenson's phosphate buffer, pH 7.4, containing 1% SLS) before the study commenced. In the pretreated dialysis membrane closed tightly from one side, 1 ml of the bilosomal dispersion (equivalent to 2 mg of LVS) was micro pipetted, and the other side was sealed by a dialysis clamp, and then the dialysis sac was immersed in a conical flask containing 100 ml of freshly prepared dissolution medium fixed and firmly closed in a water bath shaker operating at100 rpm and 37±0.5 ºC (with consideration of fulfilling the sink condition over the entire study).

At predetermined time intervals (30 min, 1, 2, 4, 8, 12, 24, 36, 48, 72 h), a fixed volume (1 ml) of sample was withdrawn and replaced with the same volume of fresh release medium. Finally, the concentration of LVS in the collected samples at each time interval was estimated using a UV spectrophotometer at 238 nm. The study was performed in triplicate (n=3), and the data was employed into the proper equation to obtain the percentage of total LVS amount released over time.

***In-vitro* release kinetics and mechanisms**

The *in vitro* drug release profiles of the fifteen developed formulations, along with the OPT LVS-BIL formula and free drug suspension, were evaluated by fitting the data to various kinetic modelsin order to determine the release mechanisms and kinetics, including zero-order (cumulative % drug released versus time), first-order (log cumulative % drug remaining versus time), and Higuchi's model (cumulative % drug released versus the square root of time). The fit quality for each model was assessed by calculating the correlation coefficient (r^2) for each formulation. The model that demonstrated the highest (r^2) value was selected as the best fit, indicating the predominant release mechanism for each formulation[26,27].

Statistical Analysis

The measurements were conducted three times and all obtained data were expressed as mean±standard deviation (SD)**.** Statistical analysis of the data was performed using one-way ANOVA. A *p*-value < 0.05 was considered statistically significant. All analyses were performed using Design Expert® software version 11 or Microsoft Excel 365 software.

RESULTS AND DISCUSSION

Quantitative Analysis of LVS

After UV Scanning, the maximum absorbance was observed at a wavelength of 238 nm, which was selected as the analytical wavelength (λ_{max}) for subsequent experiments. The same results were also observed in the work of Waris (2021)[28].

The UV spectrophotometric method used offered a reliable and precise way to quantify LVS concentration in dissolution samples.The calibration curve constructed for LVS showed a strong linear relationship between absorbance and concentration over the range of 2.5 to 20 µg/ml (R^2=[0.9998]).

The LVS concentration in the dissolution samples was determined by applying the absorbance readings to the regression equation derived from the calibration curve. This method is found to be precise, reproducible and demonstrated high specificity, as no interference was observed from the excipients used in the dissolution medium. Method validation confirmed the accuracy and linearity of the measurements across replicate samples.

Saturation solubility study of LVS

The obtained result showed that the solubility of LVS increased with time, reaching a steady state concentration after approximately 20 hours. The concentration at steady-state was found to be 2.3 mg/ml. The data indicated that the solubility of LVS was significantly enhanced in the presence of SLS, with a final solubility value of 2.3 mg/ml compared to its intrinsic solubility in pure water (0.0013 mg/ml). This enhancement can be attributed to the solubilizing effect of SLS. Since the saturation solubility of LVS in Sorenson's phosphate buffer (pH 7.4) containing 1% SLS was enhanced far more than in distilled water, it was confirmed as the best dissolution medium for the study. The obtained results were in a good agreement with the published paper by Alshora *et al*.[29].

Preliminary studies and selection of components

LVS is classified as a Class II drug (low solubility, high permeability) under the Biopharmaceutics Classification System (BCS), which reflects its low bioavailability, at 5 % or less. In fact, only 30 % of the oral dose is absorbed due to the low dissolution rates arising from the drug's lipophilic properties and extensive first pass metabolism in the liver and gut. LVS has a short half-life of 3 hours. It is best taken with meals in the evening, when the rate of endogenous cholesterol synthesis is the highest[3,30].

The bilosomal vesicular delivery, which has the ability to encapsulate both lipophilic and hydrophilic drugs, can offer an effective system to overcome those problems, as it can increase the drug solubility and rate of dissolution, as well as lymphatic transport, avoiding first-pass metabolism and resulting in increased oral bioavailability with well-established chemical and enzymatic stability compared to conventional liposomes and niosomes[31-33].

So, in order to successfully formulate a bilosomal vesicle that possess good physicochemical features along with efficient encapsulation of the drug, preliminary study was conducted to select the suitable system ingredients and their initial levels before developing the actual design.

Choice of cholesterol over phospholipid in bilosomes formulation

In this study, cholesterol was selected over phospholipids based on an extensive review of the literature. The usage of phospholipids in bilosomal-based drug delivery systems comes with notable challenges that may hinder their efficiency. One major drawback is their susceptibility to oxidation and hydrolysis, especially in formulations containing unsaturated phospholipids, which compromises their stability and reduces shelf life. Additionally, phospholipids often exhibit limited drug-loading capacity for lipophilic drugs and are prone to aggregation in aqueous environments, further reducing their effectiveness. The high production costs and variability in natural phospholipid sources also pose significant hurdles for large-scale application. These findings were established by several previous studies such as Drescher *et al*.[34], Gbian *et al*.[35], and Zhao *et al*.[36]. In contrast, cholesterol has demonstrated superior performance as a lipid component in the bilosomal formulations for delivering lipophilic drugs.

Cholesterol enhances membrane stability by reinforcing lipid packing, increasing the fluidity of the bilosomal lipid bilayer, and reducing permeability. These changes are essential for maintaining the integrity of drug encapsulation, controlling the release of the active ingredient, and preventing vesicle aggregation, ultimately improving the stability and bioavailability of the vesicles. It also increases encapsulation efficiency by providing a hydrophobic environment within the bilayer and enables controlled, sustained drug release, which is essential for achieving consistent therapeutic levels. Furthermore, cholesterol-containing bilosomes have demonstrated improved biocompatibility, extended circulation times, reduced immune recognition, and enhanced their effectiveness as drug delivery systems. Moreover, cholesterol is cost-effective, easier to handle, and less likely to undergo hydrolysis, making it a more practical choice for bilosomal formulations aimed at long-term stability and efficient drug delivery. These findings were coincided with several papers such as Ruwizhi *et al*.[9], Kaurav *et al*.[37], and Wang *et al*.[38].

These benefits highlight the advantages of cholesterol over phospholipids, making it a more reliable choice for developing efficient bilosomal formulations for lipophilic drug delivery. The effect of cholesterol as the lipid component in the bilosomal formulation was tested at three levels (12.5 mg, 10 mg, and 7.5 mg).

The obtained results showed that increasing the cholesterol concentration led to a marked increase in vesicle size, with minimal to no change the drug entrapment efficiency or the zeta potential. These results observed in Table 4 suggest that while cholesterol concentration impacted vesicle morphology, it did not greatly affect drug encapsulation capacity or vesicle stability.

Table 3: Characterization results of different preliminary developed LVS loaded bilosomal formulations.

Batch	EE %	Characterization (dependent variables)		
		Vesicle size (nm)	PDI	Zeta potential (mV)
F1	83.39±8.78	1403.00±62.386	0.600±0.06	-74.0±2.07
F2	97.54±1.32	534.00±10.209	0.227±0.05	-76.2±2.63
F3	97.95±2.1	354.07±4.574	0.372±0.04	-61.8±2.14
F4	98.81±1.12	359.10±11.241	0.325±0.03	-54.1±1.10
F5	97.87±1.35	798.67±70.627	0.383±0.06	-76.5±4.15
F6	98.30±0.78	523.33±14.230	0.464±0.01	-72.1±2.20
F 7	98.37±0.98	297.17±4.384	0.265±0.01	-67.0±5.09
F 8	96.74±1.58	246.57±2.159	0.22±0.02	-72.2±2.42
F9	96.48±1.93	526.30±11.505	0.524±0.01	-65.2±3.13
F10	96.59±1.82	424.97±5.519	0.218±0.01	-88.0±2.51
F11	98.66±0.98	458.43±5.313	0.442±0.01	-63.8±3.87
F12	97.98±0.16	426.4±14.886	0.516±0.10	-79.2±2.76
F 13	98.91±0.11	655.67±12.745	0.474±0.02	-66.2±1.39
F 14	98.18±0.79	543.13±9.665	0.367±0.04	-84.1±1.97

Surfactant Selection (Span 80 vs. Span 60)

In the preliminary study for the development of drug-loaded bilosomes, two surfactants were evaluated. Span 60, a solid surfactant with a hydrophilic-lipophilic balance value (HLB) of 4.7, was selected for its ability to provide stability to bilosomal formulations by forming a bilayer structure with the drug. In contrast, Span 80, a more hydrophobic surfactant (HLB=4.3), was considered for its potential to improve the encapsulation efficiency of lipophilic drugs and its ability to form vesicles with controlled release properties. The evaluation results of Span 80 and Span 60 at three different surfactant-to-lipid ratios (high: 9:1, medium: 8:2, low: 6:4) showed that Span 80 consistently provided higher drug encapsulation efficiency % (EE%) compared to Span 60, with a minimal difference (~2%). However, span 80 had a notable impact on reducing vesicle size relative to Span 60 that is a clearer response seen in the results of F1 relative to F2 as it is listed in Table 4. These results are in a good agreement with those reported by Kato *et al*.[39], and Elasied *et al*.[40].

Bile Salt Selection (SDC vs. STC)

Bile salt inclusion in the lipid bilayer of vesicles offer several advantages as it helps in protecting the vesicles and the entrapped drug from the harsh gastrointestinal environment, promotes the drug vesicles permeability through the biological membranes, including the intestinal membrane, and imparts negative charge, by which vesicles gain good storage stability, enhanced transport via the lymphatic pathway by increased uptake by M-cells in the Peyer's patches, and avoidance of first-pass metabolism (intestinal and hepatic)[31,41-45].

So, furthermore two bile salts, sodium deoxycholate (SDC) and sodium taurocholate (STC), were evaluated for their role in improving the solubility of the drug and enhancing the stability of the bilosomal vesicles. Sodium deoxycholate, a more hydrophobic bile salt, was chosen for its ability to disrupt lipid bilayers, thereby promoting better drug loading. In contrast, sodium taurocholate, a more hydrophilic bile salt, was considered for its potential to increase membrane fluidity and provide a more stable environment for drug entrapment. These findings were conveyed by many literatures such as Kaurave *et al*.[9], and Naji *et al*.[46]. The two bile salts, were tested at three levels (high: 5 mg, medium: 3 mg, low: 1 mg). Results showed that SDC significantly reduced the vesicle size in comparison to STC, while STC helped to stabilize the formulation, providing better zeta potential values but had a less pronounced effect on vesicle size. This effect is clearly seen in the results of F1 relative to F9 and F2 relative to F10 as listed in Table 4.

Table 4: Characterization results of the fifteen Box Behnken developed LVS loaded bilosomal formulations.

Batch	Dependent variables (Responses)				
	Y1: EE (%)	Y2: VS (nm)	Y3: ZP (mV)	PDI	Y4: *In-vitro* % drug release
F 1	98.81±0.22	368.17±5.980	-63.80±1.873	0.440±0.008	38.67±0.018
F 2	98.73±0.12	213.47±4.428	-66.10±3.360	0.237±0.021	31.41±0.015
F 3	99.09±0.08	278.90±3.747	-59.17±3.785	0.495±0.028	37.41±0.024
F 4	98.77±0.17	254.53±3.970	-66.60±2.931	0.322±0.027	39.31±0.026
F 5	98.66±0.19	225.07±2.011	-66.27±2.765	0.323±0.025	43.10±0.029
F 6	98.76±0.24	273.53±3.754	-63.80±3.612	0.274±0.013	40.17±0.028
F 7	98.43±0.21	262.67±3.669	-66.63±1.504	0.287±0.032	43.27±0.024
F 8	98.50±0.31	248.17±0.379	-65.80±3.580	0.313±0.022	38.64±0.011
F 9	98.47±0.29	402.40±5.074	-72.53±2.802	0.422±0.033	32.00±0.024
F10	98.80±0.09	389.43±0.611	-67.10±3.844	0.41±0.032	40.60±0.016
F11	98.83±0.12	299.57±5.054	-64.87±9.745	0.317±0.046	36.08±0.025
F12	98.78±0.23	246.53±3.683	-67.23±3.763	0.218±0.006	31.06±0.047
F13	98.33±0.32	307.67±4.669	-63.07±3.828	0.272±0.013	31.98±0.063
F14	98.75±0.15	291.77±4.801	-65.80±3.201	0.230±0.017	19.89±0.049
F15	98.54±0.24	256.70±6.670	-64.97±1.531	0.466±0.020	39.03±0.019

All values expressed as mean ± SD (n=3).

SDC, being more hydrophobic, is known to enhance solubilization and has been utilized in the development of bilosomes to enhance the delivery of lipophilic compounds by forming stable micelles. This property is crucial in formulations where lipids need to be solubilized in the aqueous phase. Its incorporation into bilosomal formulations offers several advantages like, A) enhanced stability since, SDC contributes to the stability of bilosomes, potentially surpassing that of conventional liposomes. B) Improved solubility, by increasing membrane flexibility, SDC enhances the solubility of highly lipophilic drugs within the bilosomal membrane. C) Biocompatibility and biodegradability, SDC inclusion into bilosomes bilayer offer high biocompatibility and biodegradability, with minimal toxicity. These properties make SDC a valuable component in bilosome formulations aimed at improving the delivery and bioavailability of lipophilic drugs like LVS. In contrast, STC, though effective in some systems, is more hydrophilic and may not facilitate the same level of lipid solubilization or micelle formation, that is why STC is excluded despite its relatively good performance in this study. These exceptional characteristic properties of SDC were recognized by many previous studies such[9,47,51].

Physicochemical characterization of the prepared LVS-loaded bilosomes

Entrapment Efficiency Percentage EE (%)

The fifteen bilosomal formulations (F1-F15) demonstrated successful encapsulation of LVS, exhibiting high entrapment efficiencies (EE%) ranging from 98.33±0.32% (F13) to 99.09±0.12% (F3) as shown in Table 4. This indicates the effectiveness of the chosen formulation method in incorporating the drug within the bilosomal vesicles, which is consistent with those results reported by Min *et al.*[52].

Vesicle size (VS)

All formulations produced vesicles within the desirable nano-size range, with sizes varying from 213.47±4.428 nm (F2) to 402.40±5.074 nm (F9) as shown in Table 5. This nano-scale size is crucial for enhanced drug delivery and improved bioavailability. The polydispersity index (PDI) values, ranging from 0.218±0.006 (F12) to 0.495±0.028 (F3) as shown in Table 5, suggest a relatively uniform size distribution within most formulations, although some formulations (e.g., F3, F9, F15) exhibit slightly higher PDI values, indicating potential heterogeneity that may have arisen from improper homogenization, which aligns with the findings reported by Zhou *et al.*[25], and Ammar *et al.*[53].

The zeta potential (ZP)

The zeta potential (ZP) values for all formulations were negative, ranging from -59.17±3.785 mV (F3) to -72.53±2.802 mV (F9) as shown in Table 5. These high negative ZP values suggest good stability due to strong electrostatic repulsion between vesicles, minimizing aggregation. Similar findings were reported by Mahmood *et al.*[54].

***In-vitro* release of LVS from different bilosomal formulae**

Finally, the *in vitro* drug release studies over 72 hours in Sorenson's phosphate buffer (pH 7.4) containing 1% SLS revealed a sustained release profile for all formulations, ranging from 19.89±0.049% (F14) to 43.27±0.024% (F7) release as shown in Table 5.

Considering the cumulative *in-vitro* release profiles of LVS from the bilosomal formulations (F1 - F15) after 72 hours, as illustrated graphically in Figure 1- Figure 3, the following results were observed:

Among the first group formulations (F1–F5) shown in Figure 1, F5 exhibited the highest release percentage (43.10±0.029%), followed closely by F4 (39.31±0.026%) and F1 (38.67±0.018%). F2 showed the lowest release in this group (31.41±0.015%). In the second group formulations (F6–F10) shown in Figure 2, F7 demonstrated the highest release (43.27± 0.024%), while F9 had a notably lower release (32.00± 0.024%). Among the third group formulations (F11–F15) shown in Figure 3, F14 showed the least drug release (19.89±0.049%), suggesting a more sustained release pattern, whereas F15 reached a comparatively higher value (39.03±0.019%). Overall, the variation in release percentages across formulations highlighted the influence of formulation composition on drug release behavior, with F5 and F7 were identified as the most promising in terms of drug release after 72 hrs. This sustained release is a desirable characteristic for drug delivery systems, as it can prolong drug action and reduce its dosing frequency.

Analysis of the Box-Behnken surface design and the influence of different independent variables on each response

Response 1: Entrapment Efficiency (EE%)

The ANOVA results for entrapment efficiency % showed that the Quadratic model is not statistically significant (F-value=3.30, *p*-value=0.1008), indicating that, although the model captures some variance in entrapment efficiency, the likelihood of observing an F-value of this magnitude due to random noise is relatively high (10.08%). However, certain individual factors like, the amount of surfactant (Span 80) and bile salt (SDC), exhibited significant effects on entrapment efficiency.

Surfactant amount (Span 80) was found to have a significant influence on entrapment efficiency %, with a *p*-value of 0.0336. This suggests that, surfactants like Span 80 reduce the interfacial tension between the aqueous and lipid phases. This finding aligns with previous studies that demonstrated an increase in entrapment efficiency with higher surfactant concentrations, likely due to better stabilization of the bilosomal vesicle and reduction in aggregation[27,40]. Bile salt amount (SDC) also showed a significant effect on entrapment efficiency (*p*-value=0.0325). Bile salts are known for their role in improving drug solubilization and stability within vesicles, making them effective in enhancing drug entrapment. The significant impact of bile salt concentration suggests that higher bile salt levels likely facilitated the solubilization of the hydrophobic drug, which could lead to better encapsulation within the bilosomes. This result is consistent with previous findings reported in previous studies which indicate that bile salts can increase the capacity of bilosomal formulations to encapsulate poorly water-soluble drugs[25,55,56].

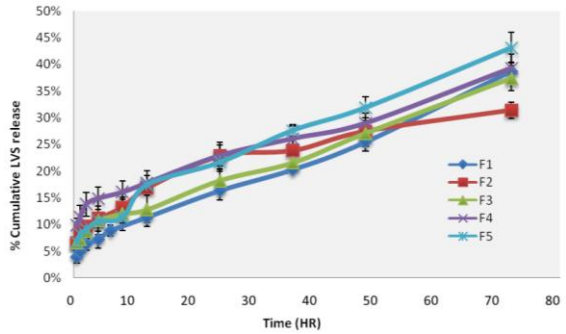

Figure 1: The cumulative release % of LVS from F1, F2, F3, F4, and F5 bilosomal formulations.

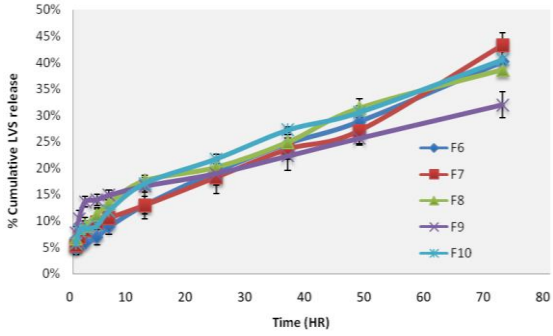

Figure 2: The cumulative release % of LVS from F6, F7, F8, F9, and F10 bilosomal formulations.

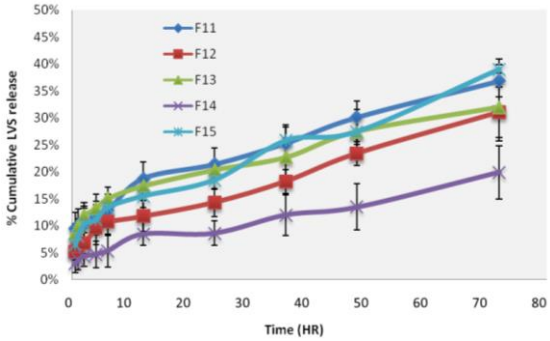

Figure 3: The cumulative release % of LVS from F11, F12, F13, F14, and F15 bilosomal formulations.

Interestingly, the lipid amount (cholesterol) did not show a significant influence on entrapment efficiency (*p*-value=0.4466). Lipid content is crucial for bilosome structure and stability; however, in this study, varying lipid concentrations within the selected range did not significantly impact drug encapsulation. This finding suggests that, within the studied range, lipid concentration may not be the most critical factor for improving drug entrapment compared to surfactants and bile salts.

The interaction terms (AB, AC, BC) and quadratic terms (A^2, B^2, C^2) were found to be non-significant (*p*-value>0.05), indicating that the independent variables (surfactant, lipid, and bile salt) did not exhibit complex interactive or nonlinear effects on entrapment efficiency. This implies that optimizing these factors individually may be sufficient for achieving optimal entrapment efficiency without the need to consider their combined or higher-order interactions.

The lack of fit was not significant (*p*-value=0.6990), suggesting that the model provided an adequate fit for the experimental data and was not significantly influenced by random error. This outcome indicates that the model is reliable for predicting entrapment efficiency based on the selected factors.

3D Response surface analysis

The response surface plots help confirm the conclusions drawn from the statistical analysis and provide a clear graphical representation of the model's predictions. The 3D response surface plots which illustrated in Figure 4, were generated to visualize the relationship between the independent variables (surfactant amount, lipid amount, and bile salt amount) and entrapment efficiency. These plots confirm the significant effects of surfactant and bile salt concentrations on entrapment efficiency, as shown by the increasing drug encapsulation with higher surfactant and bile salt concentrations. The minimal impact of lipid content is also evident in the plots, where changes in lipid concentration did not notably affect entrapment efficiency.

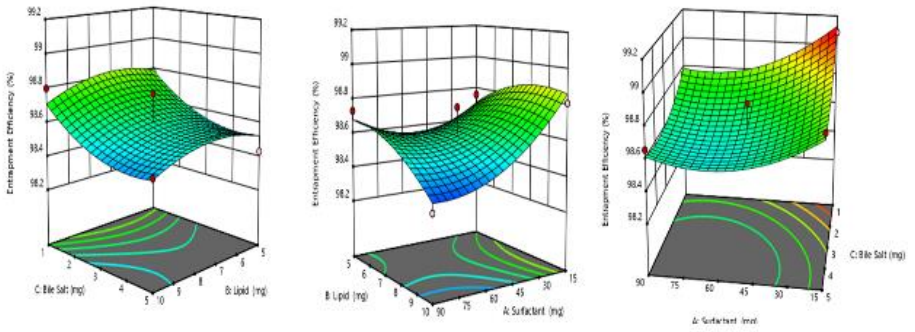

Figure 4: The 3D response surface plots demonstrate the relationship between the independent variables (surfactant amount, lipid amount, and bile salt amount) and % entrapment efficiency.

Response 2: Vesicle Size

The ANOVA results for vesicle size indicate that the model is significant (F-value=10.71, *p*-value=0.00888), suggesting that the combination of factors considered in the model has a meaningful impact on vesicle size.

Surfactant amount (Span 80) was found to significantly influence the vesicle size, with a *p*-value of 0.0295, confirming that the concentration of surfactant plays an important role in the formation of vesicles. Span 80 amount is crucial in stabilizing the vesicle membrane and controlling the size of vesicles by reducing the interfacial tension between aqueous and lipid phases. The significant effect of surfactant concentration is consistent with findings from other previous studies, which showed that increasing surfactant levels often leads to smaller, more stable vesicles by improving the encapsulation efficiency and reducing aggregation[55,57].

The lipid amount (cholesterol), with a highly significant *p*-value, was identified as the most influential factor affecting vesicle size. Cholesterol is known for its ability to stabilize lipid bilayers by enhancing membrane rigidity, which can influence vesicle formation. Higher lipid content typically leads to an increase in the vesicle size due to the greater availability of lipid molecules to form the bilayer structure, promoting larger vesicles. This finding is consistent with those reported by Zafar *et al.*[22], and Hegazy *et al.*[25], which have demonstrated that lipid content significantly impacts the size and stability of vesicles.

The quadratic term for lipid amount (B^2) was also found to be statistically significant (*p*-value=0.0103), indicating a nonlinear effect of lipid concentration on vesicle size. This suggests that at higher lipid concentrations, vesicle size increases disproportionately, likely due to changes in the packing and fluidity of the lipid bilayer. Such behavior has been also observed in similar lipid-based systems such as Alhakamy *et al.*[55], and Zafar *et al.*[58], where excessive lipid content leads to larger vesicle structures due to the enhanced bilayer organization. In contrast, bile salt concentration (SDC) was not found to significantly influence vesicle size (*p*-value=0.7905). Bile salts are typically involved in enhancing drug solubilization and absorption in bilosomes, but their effect on vesicle size in this formulation appears minimal. This lack of significance may be due to smaller amount range used that can hinder its effect pronounced at higher concentration, and its primary role in aiding drug encapsulation rather than directly influencing vesicle size.

Furthermore, none of the interaction terms (AB, AC, BC) were found to significantly affect vesicle size (*p*-value>0.05), indicating that the effects of surfactant, lipid, and bile salt concentrations on vesicle size are independent of each other. This finding is consistent with the assumption that the individual components do not interact in complex ways that influence vesicle size, at least within the studied concentration range.

The lack of fit was found to be significant (F-value=37.98, *p*-value=0.0258), which suggests that the model does not fully capture the variability in the experimental data. A significant lack of fit implies that other factors, such as preparation methods, temperature, or additional stabilizers, could contribute to the observed variance in vesicle size and should be considered in future models.

3D Response Surface Analysis

The 3D response surface plots presented in Figure 5 provide a clear visual representation of the effects of surfactant (Span 80), lipid (Cholesterol), and bile salt (SDC) amounts on vesicle size, confirming the conclusions drawn from the statistical analysis. An increase in surfactant concentration leads to a decrease in vesicle size, which aligns with the significant effect observed in the ANOVA. Conversely, lipid concentration exhibits a pronounced positive effect, with vesicle size increasing at higher levels, reflecting both its significant *p*-value and quadratic influence. In contrast, bile salt amount shows minimal impact on vesicle size, aligning with its non-significant effect. These plots offer a comprehensive and intuitive understanding of the factor interactions and their influence on vesicle size.

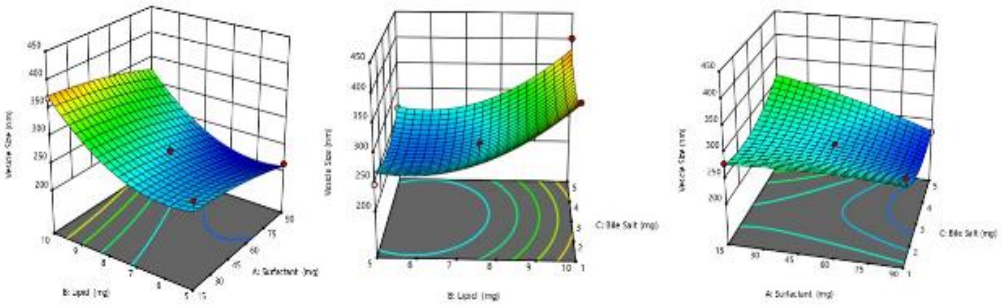

Figure 5: The 3D response surface plots demonstrate the relationship between the independent variables (surfactant amount, lipid amount, and bile salt amount) and vesicles size.

Response 3: Zeta Potential

The ANOVA results for zeta potential revealed that the quadratic model is not significant (F-value=3.19, *p*-value=0.1072), suggesting that the combination of factors considered in the model does not significantly influence the zeta potential. With a *p*-value greater than 0.05, it indicates that the variation in the zeta potential is not sufficiently explained by the levels of factors under study. The amount of surfactant (Span 80) has a *p*-value of 0.1202, indicating that it does not significantly affect the zeta potential. However, it is important to note that surfactants generally play a role

in stabilizing the vesicles by imparting a negative charge to the surface, which enhances the colloidal stability and prevents aggregation. The slight effect observed here may be attributed to the surfactant's role in improving the electrostatic repulsion between particles, leading to a more stable formulation. This effect was seen and established in a previous study[59].

The lipid amount (cholesterol) had a non-significant effect on the zeta potential (*p*-value=0.6162). Cholesterol typically stabilizes the lipid bilayer by increasing membrane rigidity, but it does not appear to significantly influence the surface charge within the concentration range studied here. This finding suggests that the zeta potential might not be highly dependent on the lipid concentration in this specific formulation.

The bile salt concentration (SDC) had a marginal effect (*p*-value=0.0849), which is slightly above the 0.05 threshold, suggesting that SDC may influence the zeta potential and its effect not captured by model due to the narrow levels used (1-5 mg). This aligns with previous research studies where bile salts are main component found to impact on the electrostatic properties of lipid-based vesicles due to its anionic nature[25,27,60]. None of the interaction terms (AB, AC, BC) were significant (*p*-values>0.05), suggesting that the effects of surfactant, lipid, and bile salt amounts on the zeta potential are independent of each other. This finding supports the idea that the zeta potential is not significantly influenced by complex interactions between these factors in the concentration ranges tested. The quadratic term for surfactant (A^2) was found to be significant (*p*-value=0.0254), indicating a nonlinear effect of surfactant concentration on zeta potential. As surfactant concentration increases, the zeta potential becomes more negative, likely due to the enhanced adsorption of surfactant molecules to the vesicle surface, increasing the electrostatic repulsion between particles and thus stabilizing the vesicular system. This finding is consistent with previous studies indicating that higher surfactant concentrations tend to improve stability by enhancing the negative surface charge[59].

The quadratic terms for lipid (B^2) and bile salt (C^2) amounts were not significant (*p*-value=0.2053 and 0.2565, respectively), indicating that changes in lipid and bile salt concentrations did not exert nonlinear effects on the zeta potential within the studied concentration ranges. The R^2 value of 0.8517 indicates that the model explains 85.17% of the variability in the zeta potential data, but the lower Adjusted R^2 of 0.5848 suggests some unexplained variance, possibly due to unmeasured factors. The negative Predicted R^2 of -1.2081 indicates the overall mean might be a better predictor than the model, highlighting potential limitations in capturing all influencing factors. However, the Adequate Precision of 7.7331, above the threshold of 4, confirms an adequate signal-to-noise ratio, meaning the model can still be useful for exploring trends in zeta potential.

Besides the well-established role of bile salts in conferring negative charge to the formulated bilosomes, surfactant concentration plays a pivotal role in controlling the zeta potential, with higher surfactant levels leading to more stable vesicles through increased electrostatic repulsion. These findings underline the importance of optimizing surfactant and lipid concentrations to achieve formulations with improved colloidal stability and potential for enhanced drug delivery.

3D Response Surface Analysis

The 3D response surface plots illustrated in Figure 6, provide a visual representation of the effects of surfactant, lipid, and bile salt amounts on the zeta potential. The plot shows that surfactant amount has the most pronounced effect on the zeta potential, with higher surfactant concentrations leading to more negative values of zeta potential, enhancing the electrostatic stability of the vesicles. In contrast, lipid and bile salt amounts show minimal effects on the surface charge, which corresponds to the non-significant *p*-value observed for these factors in the ANOVA analysis. These plots offer a comprehensive understanding of how each factor contributes to the stability of the bilosomal formulation.

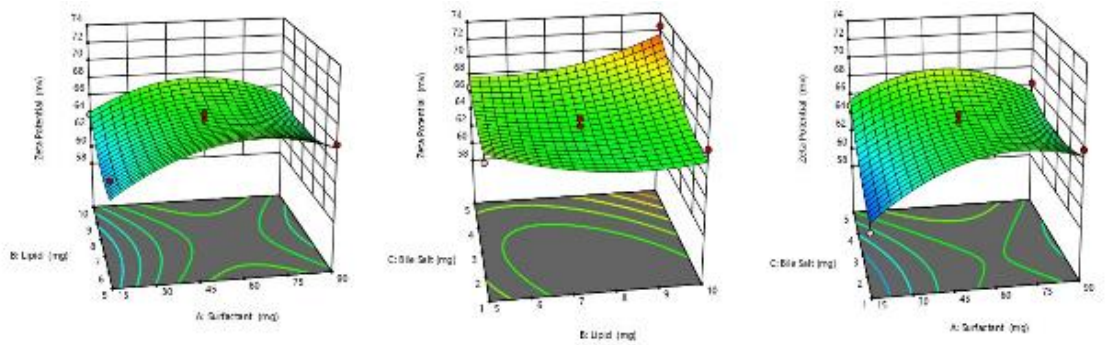

Figure 6: The 3D response surface plots demonstrate the relationship between the independent variables (surfactant amount, lipid amount, and bile salt amount) and zeta potential.

Response 4: *In-vitro* release after 72 hours

The ANOVA results for *in-vitro* drug release after 72 hours, indicate that the quadratic model is significant (F-value=5.79, *p*-value=0.0338). This suggests that the combination of factors studied has a notable influence on the release profile. The low *p*-value of 0.0338 demonstrates that the model explains a significant portion of the variability in drug release at 72 hours. However, it is important to note that some terms in the model are more significant than others.

The amount of surfactant (Span 80) has a significant effect on the *in-vitro* release (*p*-value=0.0283). Surfactants are crucial for improving the solubility of lipophilic drugs and enhancing drug release. The significant effect of surfactant concentration on the release profile likely arises due to its role in membrane fluidity and drug dissolution at the interface between the lipid bilayer and the aqueous medium. The increase in surfactant concentration relative to cholesterol likely leads to faster drug release by increasing the porosity of the vesicle membrane, thus facilitating drug diffusion into the surrounding medium. These findings align with those reported by several previous studies such as Khalil *et al*.[61].

The lipid amount (cholesterol) does not significantly influence the drug release (*p*-value=0.7668). Cholesterol is known to provide stability to the lipid bilayer, but its concentration does not necessarily correlate with the rate of drug release. Higher lipid concentrations tend to form more rigid membranes, which can potentially limit drug release. This effect was established in a previous study[57]. In the present study, the lack of significant effect suggests that the lipid concentration used does not alter the release profile to a large extent. The bile salt concentration (SDC) had a significant effect on the *in-vitro* release (*p*-value=0.0301). Bile salts are known to enhance the solubilization of lipophilic drugs by forming mixed micelles with the drug, which may facilitate the release of the encapsulated drug from the bilosomal formulation. Also increasing the concentration of bile salt (SDC) enhances the flexibility of the bilosomes membrane and increases the release of LVS from the formulation. The significant role of bile salts in this study aligns with their well-documented effect on drug dissolution and release kinetics in lipid-based delivery systems reported by several previous studies[57,61].

The interaction between surfactant and bile salt concentrations (AC) was found to be significant (*p*-value=0.0095), indicating that the combined effect of surfactant and bile salt concentration is important in determining the release rate. This interaction suggests that an increase in both surfactant and bile salt concentrations may lead to a more pronounced enhancement in the release of the drug, likely due to the dual action of surfactant-induced vesicle disruption and bile salt-facilitated drug permeation. This effect was also noticed and established by Khalil *et al.*[61]. Similarly, the interaction between lipid and bile salt concentrations (BC) was also found to be significant (*p*-value=0.0180). The interaction between lipid bilayer rigidity and bile salt solubilization plays a key role in modulating drug release. The findings suggest that higher bile salt concentrations can potentially counterbalance the membrane rigidity imparted by cholesterol, thereby enhancing the release rate of the encapsulated drug.

The quadratic term for surfactant (A^2) was marginally significant (*p*-value=0.1108). A significant quadratic effect would indicate that higher surfactant concentrations may non-linearly influence the release profile. The marginal effect observed here suggests that the concentration of surfactant may affect drug release in a non-linear fashion, but further studies may be needed to clarify the exact nature of this relationship.

The quadratic terms for lipid (B^2) and bile salt (C^2) were not significant (*p*-value=0.8027 and 0.2899, respectively), indicating that the effect of these parameters on the drug release profile does not follow a quadratic trend in the studied range.

The model's R^2 value of 0.9124 suggests it explains 91.24% of the variation in drug release, indicating a strong fit. However, the Adjusted R^2 of 0.7547 indicates that unmeasured factors might contribute to variability. The negative Predicted R^2 of -0.3956 suggests that the model may not fully capture all factors affecting drug release, and a higher-order model could improve predictions. Nevertheless, the Adequate Precision value of 9.7465, well above the threshold, confirms the model's adequate signal-to-noise ratio, making it useful for optimizing formulation parameters. The significant factors driving *in-vitro* release in this study were surfactant and bile salt concentrations, with a synergistic effect between these components. By optimizing these factors, formulations with enhanced release profiles and improved bioavailability for lipophilic drugs can be achieved.

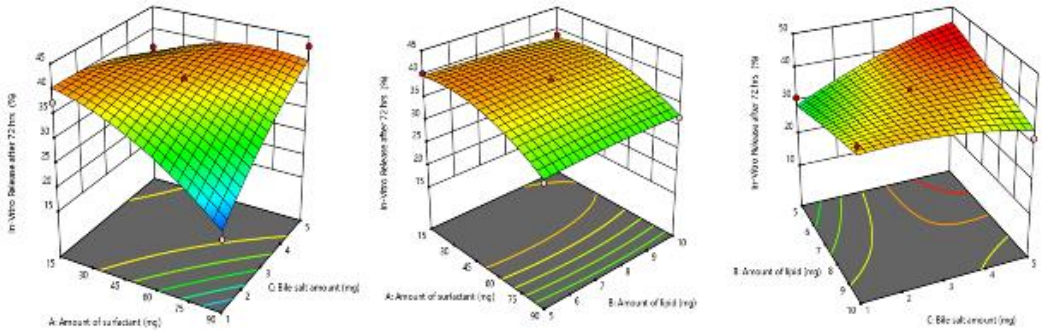

Figure 7: The 3D response surface plots demonstrate the relationship between the independent variables (surfactant amount, lipid amount, and bile salt amount) and cumulative *in-vitro* % release.

3D Response Surface Analysis

The 3D response surface plots visually depict the effects of surfactant, lipid, and bile salt concentrations on the *in-vitro* release as illustrated in Figure 7. The plots suggest that increasing surfactant and bile salt concentrations leads to higher release rates, indicating that these factors synergistically improve drug release

by enhancing the permeability of the vesicle membrane and promoting the solubilization of the drug.

***In-vitro* release kinetics and mechanisms**

The *in-vitro* release kinetics and mechanisms patterns of the fifteen design formulations, and the LVS suspension outlined in Table 5, were analyzed by fitting the data to various kinetic models in accordance with Ani Jose *et al.*[62]. The zero-order model best described the release kinetics of F1, F3, and F7 formulations as indicated by their high correlation coefficients (r) demonstrating that these formulations exhibited a constant drug release rate over time. The first-order model was the best fit for F4–F6, F8, F10–F12, F14–F15 formulations, and the LVS suspension (highest r coefficients), indicating that these formulations displayed release rates dependent on the drug concentration. Formulations F2, F9, and F13 showed the best fit to the diffusion model (highest r coefficients), suggesting that the drug release from these formulations was predominantly follow diffusion mechanisms. Overall, the release mechanisms varied significantly across the formulations, with most showing diffusion-controlled or zero-order release, while the LVS suspension exhibited a faster release pattern. The variations observed in EE%, VS, PDI, ZP, and drug release among the fifteen formulations highlight the impact of the different formulation parameters, laying the foundation for future optimization of the LVS bilosomal delivery system.

Table 5: Release kinetics and mechanisms of drug release for 15 design formulations, and LVS suspension.

Batch	Constant	Value	Mechanism of Release
F1	r	0.99683	Zero Order
F2	r	0.99451	Diffusion
F3	r	0.99594	Zero Order
F4	r	-0.9917	First Order
F5	r	-0.9965	First Order
F6	r	-0.9984	First Order
F7	r	0.99365	Zero Order
F8	r	-0.993	First Order
F9	r	0.98184	Diffusion
F10	r	-0.9941	First Order
F11	r	-0.9919	First Order
F12	r	-0.9931	First Order
F13	r	0.99543	Diffusion
F14	r	-0.9884	First Order
F15	r	-0.9919	First Order
LVS Suspension	r	0.71737	Diffusion

Limitation of the study

The lack of the optimization step to identify the optimal bilosomal formulation and its full characterization. While promising, the absence of formulation refinement and detailed characterization may hinder the translation of these findings into clinical practice. Future studies should perform the optimization and in-vivo evaluations to better assess therapeutic effectiveness.

CONCLUSIONS

This research investigates the use of Sorenson's phosphate buffer (pH 7.4) containing 1% Sodium Lauryl Sulfate (SLS) as an effective dissolution medium for lovastatin (LVS). The study demonstrated that this medium significantly enhanced the solubility of LVS, providing a more favorable environment for drug dissolution. Furthermore, the research highlights the potential of LVS-loaded bilosomes as an innovative drug delivery system. These bilosomal vesicles exhibited high encapsulation efficiency (EE%), which is a critical parameter for ensuring adequate drug loading and therapeutic efficacy. The vesicles also displayed a nano-size range, which is beneficial for improving drug absorption and bioavailability by facilitating cellular uptake. Additionally, the bilosomes exhibited a negative zeta potential, indicative of their stability, which is essential for preventing aggregation and ensuring consistent delivery of the drug. Importantly, the LVS-loaded bilosomes demonstrated sustained release patterns, suggesting that they could offer prolonged therapeutic effects. Collectively, these findings underscore the promising potential of bilosomal formulations as an efficient and reliable drug delivery system for enhancing the solubility, stability, and bioavailability of poorly water-soluble drugs, such as LVS, thus improving their clinical effectiveness. For a comprehensive understanding of the clinical applicability of this novel approach, future studies should focus on *in-vivo* evaluations and assessments of its therapeutic efficacy.

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AUTHOR'S CONTRIBUTION

Abdelatif H. Abdelatif: writing, design, methods, experiments. **Ahmed H. Ibrahim:** data management, analysis. **Sherif Kh. Abu-Elyazid:** methods, review. **Ahmed M. Samy:** revisions, analysis. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

The accompanying author can provide the empirical data that were utilized to support the study's conclusions upon request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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