Original Research Article

The Utilization of Response Surface Methodology (RSM) In the Optimization of Diclofenac Sodium (DS) Liposomes Formulate Through the Thin Film Hydration (TFH) Technique with Involving Computational Method

Abstract:

Objectives: The objective of the current studies to enhance the formulation of DS-loaded liposomes through the utilization of RSM and involving the computation approach for their validation.

Methods: The optimization of DS-loaded liposome was conducted using RSM, focusing of 2 main key parameters including encapsulation efficiency (% EE) and In-vitro drug release (% DR) for 12 hours via involving QbD. To formulate an optimize liposome formulation utilizing a 3^2 factorial design, with the phospholipid and cholesterol (CH) concentrations being the chosen independent variables. Nine formulations of DS-loaded liposomes were prepared using the TFH technique. The % EE, drug content, and in vitro release studies were assessed utilizingan Ultra Violet (UV)-visible spectrophotometer for λ_{max}-275 nm. The evaluation includedzeta potential, vesicle characterization,particle size and polydispersity index (PDI)of the best optimizedDS formulation were evaluated. Lastly the involvement of computational tools, such as molecular dynamics simulations docking with COX-2 active site via Y385.

Results: The regression equations using RSM revealed that the phospholipid and CH molar concentration were significant variables in optimizing the percentage of %EE and percentage of drug release (% DR), with estimated coefficient values. The % EE was found to be 83.55±0.29, while the % DR was 71.22±0.34. The assumption of % DR and % EE values showed low % relative errors (PRE) of 0.069% and -0.194% respectively. The result shows that the design-developed model is appropriate for DS formulations and validates the model.

Conclusion: Investigational outcome represents the perceived responses were in related with the desired values and this represents the relationship of the RSM for optimization of % DR and % EE in DS loaded liposomal preparations.

Keywords: Optimization, Diclofenac (DS), Liposomes, Thin film hydration technique, DoE; Design of Experiments.

1. INTRODUCTION

Diclofenac sodium (DS) is a BCS class II drug. This means that it is less soluble but more permeable. The class II drugs for BCS are typically absorbed well by the body, but they may exhibit variable bioavailability due to their poor solubility. The poor solubility of DS can be overcome by formulating it in a variety of ways, such as using micronized particles, dissolving the drug in a surfactant solution, or encapsulating the drug in liposomes [1-2].

DS is a NSAID(non-steroidal anti-inflammatory drug) that is employed to cureinflammation, pain, and fever. It is accessible in a several formulations, including capsules,oral tablets, suppositories, topical gels, ointments, and injectable solutions. DS serve by blocking the synthesis of prostaglandins that is important for inflammation and pain response. It is effective for treating various disease, like: Arthritis (e.g., ankylosing spondylitis,rheumatoid arthritis,osteoarthritis), menstrual cramps, Migraine headaches, acute pain (e.g., post-operative pain, dental pain, and muscle pain). DS is generally well-tolerated, but it can cause side effects in some people [3]. The most common side effects are mild and go away on their own. However, serious side effects can occur, such as stomach bleeding and ulcers. The drug description shown in the given **Table. 1** as below followings:

Table. 1: The description of diclofenac sodium drug

Characteristic	Description
Drug class	NSAIDs
Available forms	Oral tablets, capsules, suppositories, topical gels and ointments, injectable solutions
Mechanism of action	Blocks the synthesis of prostaglandins, that is important for inflammation and pain
Uses	Treats pain, inflammation, and fever
Dosage	Depends on the condition being treated and the individual's response to the drug
Side effects	Nausea, vomiting, stomach upset, diarrhea, headache, dizziness, drowsiness (more common); stomach bleeding and ulcers, liver damage, kidney damage, heart attack, stroke (less common)
Interactions	Can interact with other medications
Pregnancy and breastfeeding	Not recommended for use during pregnancy or breastfeeding [2-4]

To increase the poorly solubility of diclofenac drug via liposome, the following steps can be followed:

- ***Choose the right lipid composition.*** The lipid composition of the liposome can have a big impact on its solubility. For DS, a combination of phosphatidylcholine, cholesterol, and dipalmitoylphosphatidylglycerol (DPPG) has been shown to produce liposomes with high diclofenac encapsulation efficiency and solubility.
- ***Prepare the liposomes.*** There are a number of different methods for preparing liposomes. A common method is to dissolve the lipids in a solvent, such as ethanol or chloroform, and then add water for formation of an emulsion. The emulsion is further sonicated or extruded to form liposomes.
- ***Encapsulate the diclofenac.*** Once the liposomes have been prepared, the DS can be encapsulated by adding it to the liposome suspension and mixing gently. The diclofenac will diffuse into the liposomes and become encapsulated in the lipid bilayer.
- ***Purify the liposomes.*** Once the DS has been encapsulated, the liposomes can be purified to remove any unencapsulated diclofenac. This can be done by dialysis, gel filtration, or ultracentrifugation [4-6].

The resulting diclofenac liposomes will have increased solubility compared to conventional DS formulations. This is because the DS is encapsulated in the lipid bilayer of the liposome, which protects it from the external environment.

Liposome drug delivery is a method of transporting drugs into the body using microscopic vesicles called liposomes. Liposomes has a lipid bilayer, which is a bilayer of phospholipids forming a spherical surface. This bilayer surrounds an aqueous core, which can be used to entrap both hydrophilic (water-soluble) and lipophilic (water-insoluble) drugs [7]. Liposome drug delivery has various benefits over traditional drug delivery methods. First, liposomes can prolong the drugs half-life asit protects the drug from degradation in the body. Second, liposomes targets drugs to specific cells or tissues. Third, liposomes can controlled the release of drugs over time. Liposomes are prepared by mixing lipids and water in a specific ratio. The mixture is then sonicated or extruded to form liposomes. Size and properties of the liposomes can be controlled by varying the lipid composition and the preparation method.

Once the liposomes are formed, the drug can be encapsulated by adding it to the liposome suspension. The drug will penetrate through the liposomes and become encapsulated within lipid bilayer. The liposomes can then be purified to remove any unencapsulated drug [6-8]. Liposomes can

be administered into the body by a different routes, like as injection, oral administration, and topical application. The route of administration depends on the intended utilization of the liposomes.

Liposome drug delivery is a versatile and promising drug delivery system. It has the capacity to enhance the safety and efficacy of many drugs.Several prior studies have investigated the efficacy of ibuprofen [9], naproxen[10-11], and Nimesulide [12, 13] in improving the bioavailability and poor water solubility of these compounds. Therefore, it is imperative to develop and DS-loaded liposomes are potentfor anticancer treatment. In the field of pharmaceutical advance technique, the optimization and development of various pharmaceutical dosage forms are influenced by a multitude of elements that impact the product assumptions [11-13].

For the using computational approaches can be used to significantly accelerate the development of liposomes for the delivery of DS. By using computational methods, it is possible to screen a large number of liposome formulations and to identify the most promising formulations for further evaluation. This can save a significant amount of time and resources.The specific computational approaches that can be used for the design and optimization of liposomes for the delivery of DS include, Molecular dynamics (MD) simulation can be used to simulate the behavior of liposomes and DS molecules at the atomic level [11-13]. This information can be used to understand the interactions between the liposomes and DS molecules, and to predict the properties of liposome formulations and docking for their predictions.

The formulation were outlined to investigate the impact of independent variables, or factors, over the dependent variable, or response/parameter, of a given formulation or process. RSM is a type of experimental design, is a highly effective tool for showing the correlation between a set of quantitative factors and a response. RSM utilizes a quadratic polynomial model to identify the optimal response. One of the key advantages of RSM is that it requires fewer experiments, which can significantly reduce the cost of analysis [12-13]. RSM is particularly utilized for plotting a plan of the response surface, identifying the optimal variable level for a response, andformula to meet specific requirements or selecting the appropriate process conditions. In this study, we aimed to optimize the diclofenac sodium formula by varying independent variables such as cholesterol (CH) mass and phospholipid (phosphatidylcholine) mass [14]. The optimal formula was determined using RSM with a 3^2 full factorial design, and the approach was adapted to achieve the desired % DR and % EE for NSAID drug.

2. MATERIAL AND METHODS

Materials: Diclofenac sodium (DS), (purity>90%), phospholipids samples (phosphatidylcholine) with purity >90%, was obtained. The solvents especially methanol and chloroform used for the formulation of liposome with DS. Potassium dihydrogen phosphate, sodium hydroxide pellets and Cholesterol (CH) are thechemical utilized for the work of analytical grade (AR) [11-15]. The chemical structure of all materials used in the formulation as per **Fig. 1** as below following:

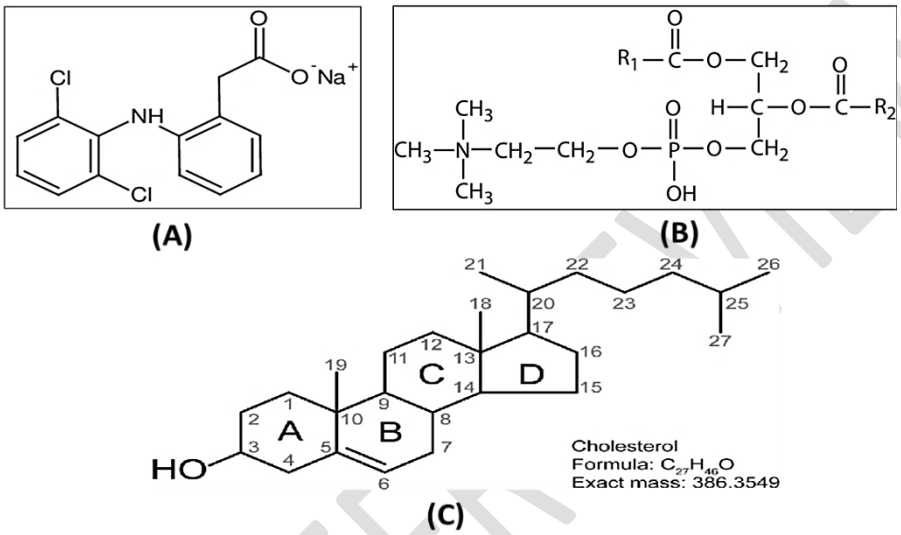

Figure. 1: The structural composition of chemicals in the formulation of liposome: (A). Diclofenac sodium (DS), (B). Phosphatidylcholine (Phospholipid) and (C). Cholesterol (CH)

Experimental studies (3^2FFD): In order to optimize the acquisition of information on product properties while minimizing the number of trials, a FFD (3^2) was employed to organize an investigation ofa linked impact of independent variables over dependent variables. The study evaluated two factors, each at three levels, along with experimental trials were conducted at all nine possible blends.The first independent variable, R1, represents the compositional amount of phospholipid, with three different levels of 1, 2, and 3 moles. Second independent variable, R2, represents the mass of cholesterol, also with three different levels of CH-1, 2, and 3 moles. These variables were picked for the liposomes. The levels of these variables were categorized for lesser level (-1), medium level (0), and higher level (+1). Entire calculations were conducted at the milligram level. The concentration of DS was kept constant at 10 µM, and the final formulation volume was maintained at 15 ml [16].The dependent variables chosen for analysis were the percent entrapment efficiency (% EE) (P1) and the % in vitro drug delivery at 12 hours (% DR) (P2). The values of the batch codes and variables can be found in **Tables 2** and**3.**

The statistical experimental design was generated and evaluated using Design Expert® DX 10.0.7.0 software, licensed version, developed by Stat-Ease Inc., MN [15-18].

Preparation of Diclofenac sodium-loaded liposomes: Theliposomes were formulated by employing the thin film hydration method (TFH). A constant concentration of 10 µM DS (molecular weight: 318.13 grams per mole g/mol.) was used for all batches, along with the desired amounts of phospholipid (phosphatidylcholine) (molecular weight: 770) and cholesterol (CH) (molecular weight: 386.67), which were solubilized in 10 ml of chloroform in a 100 ml RBF (round bottom flask). The experimental design in **Table 1** was followed for all batches. Chloroform was vaporized via a rotary vacuum evaporator and left overnight under vacuum. The resulting mixture was hydrated with 15 ml of phosphate buffer pH 7.4 for 1 hour with 10 minutes of immense vortexing [19-20]. The liposome suspension was sonicated with in the water bath at a temperature of 60 °C to lower the size of the liposomes (**Fig. 2)**.

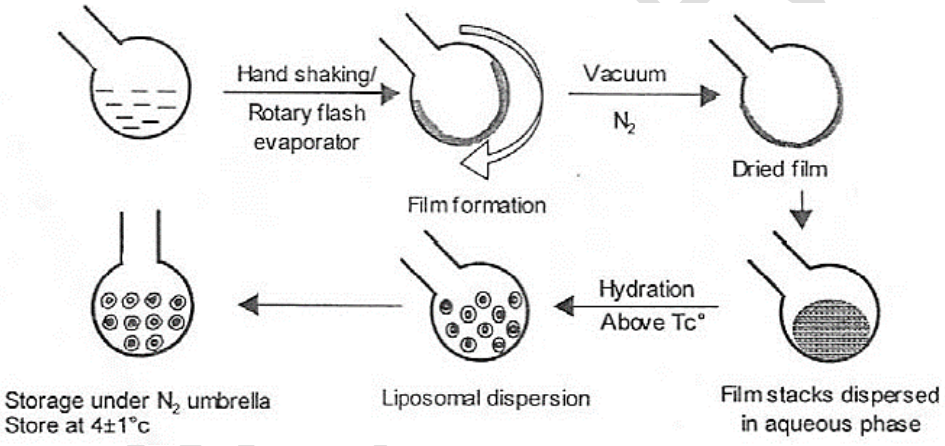

Figure. 2: The formulation of liposomes via thin film hydration techniques

Non-incorporated diclofenac sodium was broken down by ultracentrifugation for 30 minutesat 4 °C and 10,000 rpm. The supernatant was thrown out, and the DS-loaded liposomes in the precipitate were re-scattered in the needed volume of phosphate buffer pH 7.4. The resulting solution was shifted to vials to store at 4 °C [20].

Table. 2: The 3^2FFD: responses with their factor, factor levels for diclofenac sodium preparation

Factors (Independent variables)	Factor ranges employed		
	Low (-1)	**Medium (0)**	**High (+1)**
Concentration (moles) of phospholipid	**1**	**2**	**3**

(phosphatidylcholine) (R_1, w)			
Concentration (moles) of cholesterol (CH) (R_2, w)	**1**	**2**	**3**
Responses (Dependent variable)			
P_1 = % EE ; P_2 = % DR			

These are the some dependent and independent variables for the 3^2 factorial design for diclofenac liposome formulation.

3. STATISTICAL STUDY AND OPTIMIZATION OF PREPARATION USING RSM

3.1.Determination of % encapsulation efficiency (% EE): The DS preparation was purified using an ultracentrifugation technique [28, 29]. To determine the concentration of DS encapsulated, 2 ml of the vesicular dispersion was centrifuged for 1 hourat 10,000 rpm and 4 °C using a refrigerated centrifuge. The supernatant, which contained the un-entrapped drug, was withdrawn and measured using UV spectrophotometry at aλ of 275 nm. The measurements were performed against a 25:75 ratio for methanol to phosphate buffer solution (PBS) with a pH of 7.4. Every measurements were conducted in triplicate. Calibration plot was generated with mixing reference solutions of DS with a 25:75 ratio of methanol and PBS with a pH of 7.4 [20-21]. The concentration of drug encapsulated in liposomes was given by equation as below followings:

$$(\%\mathbf{EE}) = \frac{\textbf{Amount of entrapped drug}}{\textbf{total amount of drug}} \textbf{x 100}$$

The percent encapsulation efficiency (% EE) was calculated as the percentage of the available dissolved solute that was final entrapped.

3.2.Estimation of % In-Vitro drug release studied (% DR) at 12 hr.: The % DR study for PT by variousDS formulations with utilized the Franz diffusion cell apparatus, which consists of a donor and receptor section, with an effective surface area for dissolution of 2.205 cm^2. A dialysis membranewas employed and pre-treated according to the manufacturer's instructions. Once properly pre-treated, the membrane was cut to the requiredshape, sizes and staged connecting the effective surface area of the receptor and donor compartments. A 2 ml DS dispersion was put over the membrane, along with the inclusion of 20 ml of PBS (pH 7.4) containing 0.12% span 60 as the dissolution media in the receptor compartment [22]. The contents of receptor compartment were stirred at 100 rpm using a magnetic stirrer at a temperature of 37±1.0 °C.

At predetermined time intervals, 2 ml samples were extracted from the sampling port of the apparatus. These samples were appropriately diluted with clear media and the absorbance of the resultant solutions was measured with 275 nm employing an UV-visible spectrophotometer.

3.3.The vesicular morphology of liposomes: Liposomes were affixed to a slide of glass and subjected to morphological observation using a Digital Microscope following appropriate dilution. Size analysis of DS was conducted at a magnification of (×40) utilizing a calibrated eyepiece micrometers. The images were captured using the Motic Image plus 2.0 ML software that accompanies the instrument [23].

3.4.Estimation% drug content: A volume of one milliliter of suspension was extracted from the DS preparation and subsequently mixed in methanol for lysis. The resulting solution was further diluted using a 25:75 ratio of methanol to PBS with a pH of 7.4. The samples were then subjected to spectrophotometric analysis at a wavelength of 275 nm to determine the concentration of DS[24].

3.5.Evaluating of Zeta potential, particle size and Poly-dispersity index (PDI): Liposomal size was determined with a Malvern zetasizerusing dynamic light scattering. A diluted (1:100) DS suspension was taken in the sample cuvette and placed in the zetasizer. The sample was allowed to stabilize for two minutes before taking measurements. The average particle size was determined by demonstrationof the experiment three times. The zeta potential of the formulatedDS loaded preparation was obtained by a Malvern zetasizer [24-26].

4. COMPUTATIONAL APPROACH VALIDATION:

The docking pose of DS in the active site of ***cyclooxygenase-2 (COX-2***) is characterized by a number of important interactions. The carboxylate group of DS forms a salt bridge with the arginine residue Arg117, while the dichlorophenyl ring interacts with the hydrophobic residues ***Val349, Ile523, and Trp387.*** The methoxy group of diclofenac sodium also interacts with the hydrophobic residue Val524. These interactions help to anchor diclofenac sodium in the active site of COX-2, allowing it to inhibit the enzyme's activity [27].

In addition to these interactions, the docking pose of DS also allows it to interact with the tyrosine residue Tyr385. This interaction is thought to be important for the selectivity of diclofenac sodium for COX-2 over COX-1. COX-1 and COX-2 are two isoforms of the cyclooxygenase enzyme, and they play different roles in the body. COX-1 is involved in the production of prostaglandins that are important for normal physiological functions, such as protecting the stomach lining and regulating blood flow. COX-2 is involved in the production of prostaglandins that are responsible for inflammation and pain.

By interacting with Tyr385, diclofenac sodium is able to selectively inhibit COX-2 without significantly inhibiting COX-1. This helps to minimize the side effects of DS, such as stomach ulcers and bleeding [25-27]. The docking pose of diclofenac sodium in the active site of COX-2 as per **Fig. 3**as below followings:

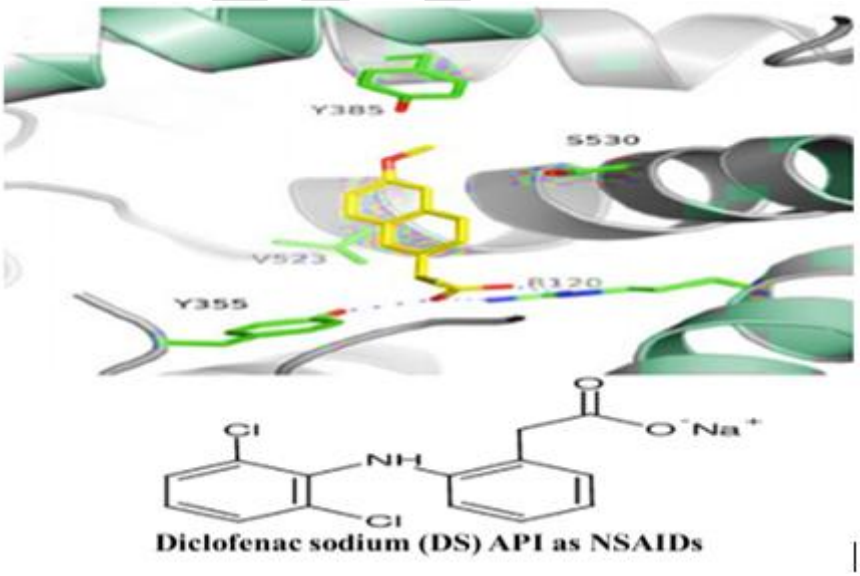

Figure. 3: The docking pose of diclofenac sodium with Y385 at COX-2 active site

The diagram shows that diclofenac sodium is well-anchored in the active site of COX-2 by a number of interactions, including a salt bridge with Arg117 and hydrophobic interactions with Val349, Ile523, Trp387, and Val524. DS also interacts with Tyr385, which is thought to be important for its selectivity for COX-2 over COX-1 [27].

5. RESULTS & DISCUSSION

5.1.Experimental data and design acquiring (3^2FFD): A FFD (3^2) was utilized to improve the preparation of DS. **Total.03 batches** of DS were prepared in accordance with the preparation variables outlined in **Table 3.** Liposome were acquire by employing the TFH technique. RSM was employed to assess the impact of the molar ratios of phosphatidylcholine and CH, as well as their interconnection, on the dependent variables of interest (% DR and % EE) [27-28]. The objective of this experiment was to identify significant factors that influence the performance of the formulation and establish their optimal levels to achieve the desired responses as presented in **Table 3.**

Table. 3: The Composition 3^2FFD with measured responses of diclofenac sodium formulation

Batches	Variable range		Variable range in real form		Response variables	
	X_1	X_2	Phospholipid (phosphatidylcholine) in moles (R_1, W)	Cholesterol (CH) in moles (R_2, W)	(% EE)±SD	(% DR)±SD For 12 hr
L1	-1	-1	1	1	78.65±1.28	40.28±1.58
L2	-1	0	1	2	64.23±0.32	42.68±0.98
L3	-1	+1	1	3	51.68±0.45	32.39±1.02
L4	0	-1	2	1	83.55±0.59	71.22±1.34
L5	0	0	2	2	75.69±1.1	62.12±0.63
L6	0	+1	2	3	63.23±1.57	66.98±1.33
L7	+1	-1	3	1	72.12±1.73	59.32±1.42
L8	+1	0	3	2	66.16±0.21	61.85±0.87
L9	+1	+1	3	3	62.89±1.22	72.92±1.19

5.2.Statistical studies with optimization of preparation employing RSM: In order to assess the quantitative impact of factors R1 and R2, as well as their respective levels ofhigh (+1), middle (0), and low (-1), on preferred responses, experimental values of flux were studied using Design Expert® DX 10.0.7.0 license version software. Models of mathematics were then employed for each response, as documented in references [25-31]. The mathematical link was produced to study response variables via multiple linear regression analysis (MLRA), namely % DR and % EE at 12 hours, which relate toindependent variables and various response are conveyed as quadratic models in the following polynomial equations:

$$P1\ (\%\ EE) = 83.44+3.19X1–8.33X2+3.99\ X1X2–9.81X12+0.25X21 \quad (1)$$

$$P2\ (\%\ DR12\ h.) = 71.22+6.79X1–4.23X2–1.39X1X2–15.39X1\ 2–4.82X21 \quad (2)$$

These equations demonstrate the effect of independent variables, specifically the molar ratio of phosphatidylcholine and CH, on dependent variables like as % EE (P1) and in vitro % DR at 12 hours (P2). The fitted quadratic models for % EE and % DR were used to draw conclusions by considering the coefficients and mathematical signs, both +ve and -ve. The correlation coefficient (R^2) of model was found to be significant, with a value of 0.9516 for response P1 (% EE) and 0.9878 response of P2 (% DR).

5.3.Response 1 (% EE): The regression analysis conducted on equation (1) for response P1 (% EE) indicated that the coefficient of β1 was (+) ve, while β2 was (-) ve. Theresult suggests that an increase in phosphatidylcholine (R1) led to an increase in % EE, but further increasing phosphatidylcholine (R1) to higher levels resulted in a decrease in % EE. Additionally, an increase in cholesterol (R2) was found to decrease % EE. This can be attributed to the fact that higher concentrations of cholesterol lead to vesicle rigidity [26] that reduces % EE. The % EE of various liposomal batches ranged from 51.68 to 83.55. %. Batch L4 (**Table 4**), which had a constituent of phosphatidylcholine: CH (2:1 molar ratio) (0,-1), exhibited the highest entrapment [30-32].

The counter plot (**Fig. 4**) and ANOVA analytical analysis (**Table. 4**) of %EE for diclofenac sodium liposome formulation as shown as below followings:

Table. 4: The list of ANOVA analytical of % EE

Source	F Value	Sum of squares	Mean squares	p-value Prob. >F
Model	21.89	829.57	165.91	0.0142
R_1–Phospholipid (PL90G)	7.70	57.72	57.72	0.0692
R_2–Cholesterol (CH)	71.36	534.68	534.68	0.0035
R1R2	5.42	40.58	40.58	0.1024
R12	26.20	196.35	196.35	0.0144
R22	0.032	0.24	0.24	0.8693
Cor–total		852.05		

ANOVA was used to determine the model's significance, and the model's response F-value (P1) (21.89) from the ANOVA data showed that model is significant as represented in **Table 4.**

The counter plot shown as below **Fig. 4**followings**:**

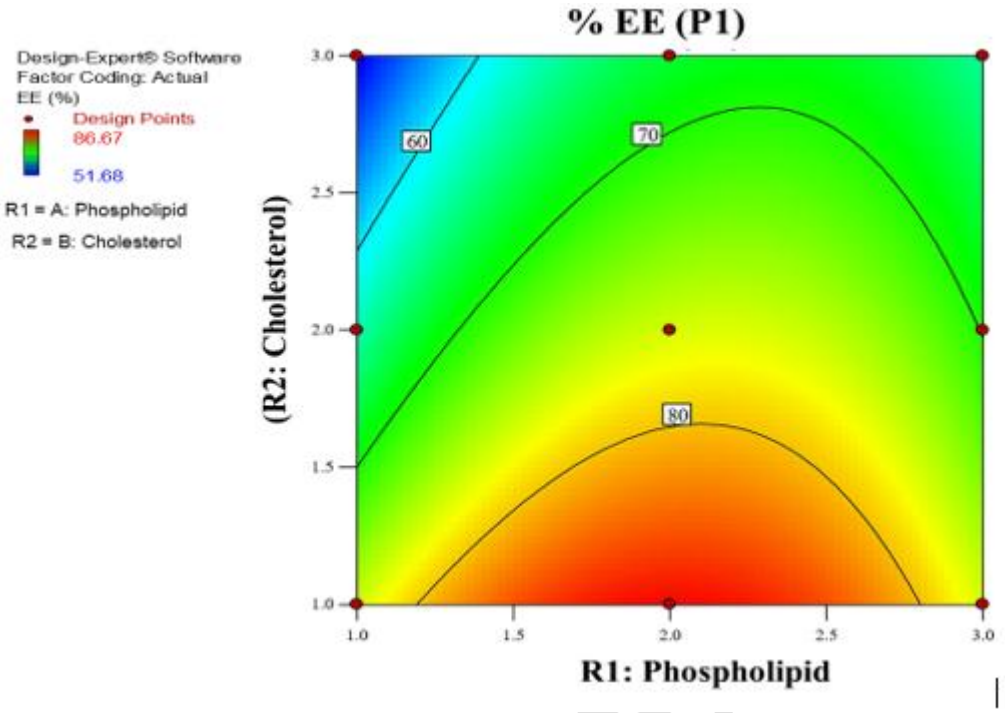

Figure. 4: The Counter plot representing the results of cholesterol (CH) (R_2) phospholipid (phosphatidylcholine) (R_1) on % EE (P_1) of DS

The response surface plot for the % EE as below as following **Fig. 5:**

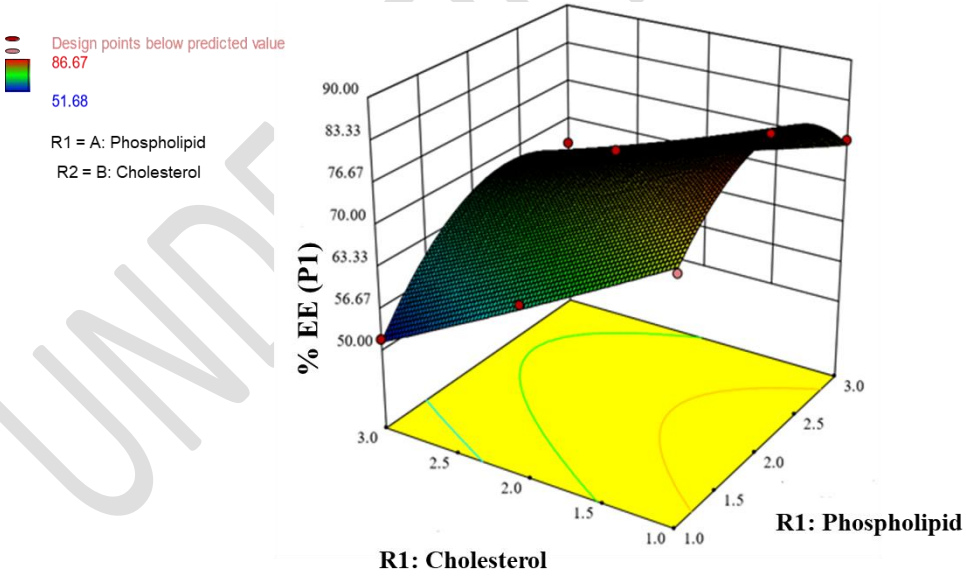

Figure. 5: Response surface plot representing the result of cholesterol (CH) (R_2) and phospholipid (phosphatidylcholine) (R1) on % EE (P1) of DS liposome

5.4.Response 2 (%DR): The impact of drug delivered at 12 hours (% DR) (P2) was given statistically significant (P<0.05) according to the analysis of variance (ANOVA). The polynomial equation (7) indicated that the coefficient of β1 was (+)ive and β2 was (-)ive, suggesting that an increase in phosphatidylcholine (R1) led to an increase in % DR, while an increase in cholesterol (R2) resulted in a decrease in % DR. The % DR elevated with higher concentrations of lipid, but reached a point where the release was slowed down, and at higher levels of cholesterol, the release was decreased. This can be attributed to the fact that higher levels of cholesterol make the lipid bilayers extra rigid, thus impeding liberation of the drug. This was notable from vesicles with greater cholesterol concentrations, which represented approximately 50% release, except for the L6 preparation [31, 32]. The L4 preparation, with a constituent of phosphatidylcholine: CH (2:1 molar ratio) (0,-1), exhibited a % DR of 66.98. % at 12 hours (**Table 3**). At lesser concentrations of cholesterol and phospholipid, the drug liberation was minimal due to the preparation of a stagnant layer [26].

The counter plot (**Fig. 6**) and ANOVA analytical analysis (**Table. 7**) of %EE for diclofenac sodium liposome formulation as shown as below followings:

Table.5: The list of ANOVA analytical of % DR at 12 hr.

Source	F Value	Sum of squares	Mean squares	p-value prob>F
Model	24.39	948.33	189.67	0.0109
A-Phospholipid	38.70	276.62	276.62	0.0084
B-Cholesterol	18.02	128.81	128.81	0.0239
AB	1.06	7.59	7.59	0.3786
A^2	65.41	467.57	467.57	0.0040
B^2	9.48	67.74	67.74	0.0542
Cor Total		969.78		

With ANOVA analysis, the model F-value of response (P2) (24.39) represents the model is significantly mentioned in **Table 5.**The counter plot (**Fig. 6)**% DR as below followings:

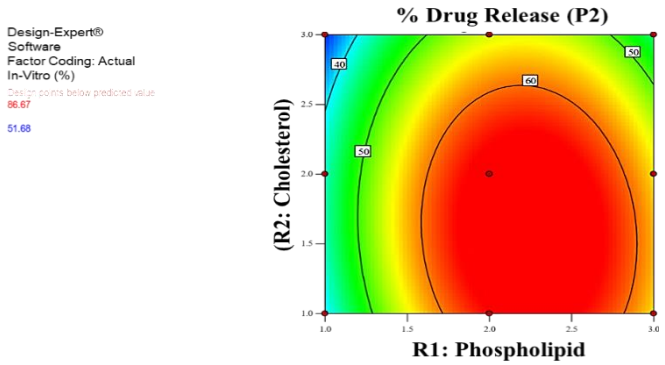

Figure. 6: Counter plot representing the result ofand cholesterol (CH) (R2) and phospholipid (phosphatidylcholine) (R1) on % DR for 12 h (P2) of PTL

The response surface plot for the % EE as below as following **Fig. 7:**

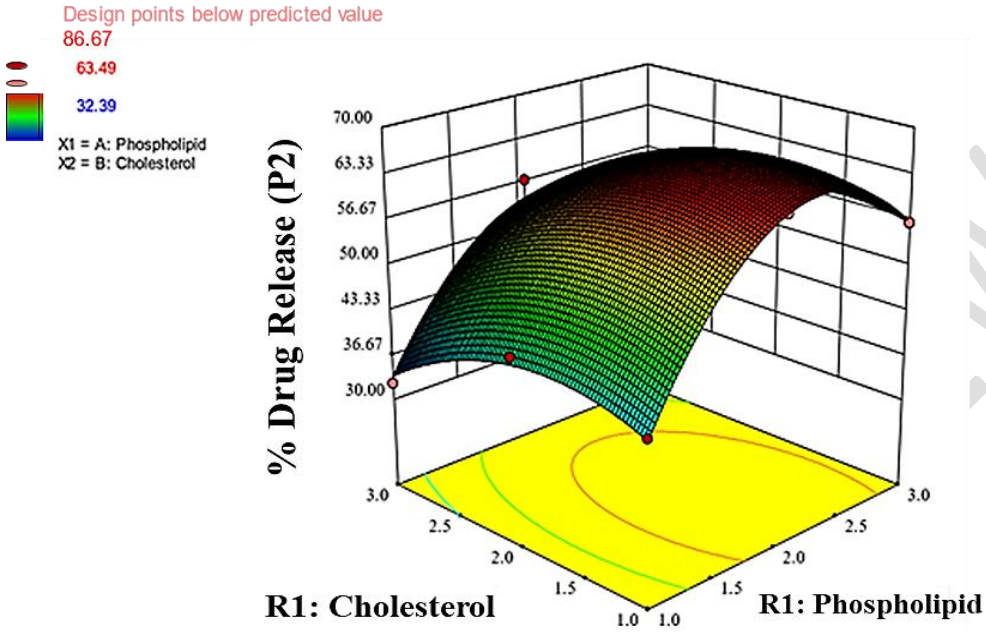

Figure. 7: Response surface plot representing the result of cholesterol (CH) (R2) and phospholipid (phosphatidylcholine) (R1) on % DR for 12 h (P2) of DS liposome

5.5.Desirability and overlay plot: The objective of liposomal preparationthe optimization is typically to identify the optimal ranges of variables that influence desirability (**Fig. 8)** responses, in order to produce a strong product with top-notch qualities. Throughout the optimization process, all measured responses that could impact the product's quality were carefully considered. Specifically, the criteria for maximum % EE and % DR at 12 hours were established [33-35]. By The best value was found by merging each answer criterion using an overlay plot. (**Fig. 9**).

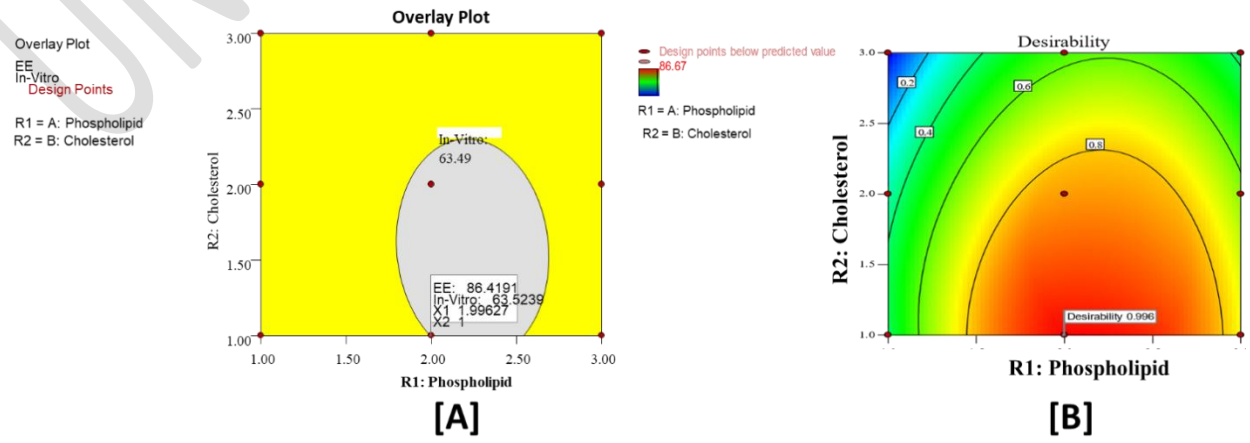

Figure. 8 &9: The overlay plot a desirability plot of DS liposome; [A]. Overlay plot and [B]. Desirability Plot

5.6.To validatethe RSM obtained Results: To assess optimization capabilities of models generated through the RSM (3^2FFD), a DS preparation was formulated utilizing the optimal technique variable settings where R1 and R2 were 2:1. The resulting P1 (% EE) and P2 (% DR at 12 h) responses obtained from the predicted models and experimental model are presented in Table 8. The percent relative error (PRE) was calculated using **Equation 4.** The PRE values for P1 (% EE) and Y2 (% DR at 12 h) were determined to be (-0.189) and (0.069), respectively. The maximum PRE value was (-0.189); however, all values were found to be <2%, confirming the suitability of the experimental study [34-36]. This outcome demonstrate good relationship between the preparation properties and theoretical properties.

Table. 6: Verification of experimental and predicted diclofenac sodium batch formulation

Response	Experimental values	Predicted value	% Correlative error (PRE)
Y_1 (% EE)	86.66±0.66	86.418	–0.291
Y_2 (% DR at 12 h)	63.48±1.22	63.526	0.059

5.7.Percent drug content, vesicle morphology, PDI and particle size of advanced DS preparation: The latest diclofenac sodium formulation's vesicle morphology was studied using a digital microscope. As seen in Fig. 10, the liposomes had a spherical shape and a smooth surface. The percentage of medication in the liposomal form estimated to be 98-1.0% (n = 3,mean SD). The near 100% drug contains means that no substance was lost during production, and the high percentage signifies that the DS was evenly distributed among the vesicular dispersions. [37-39].

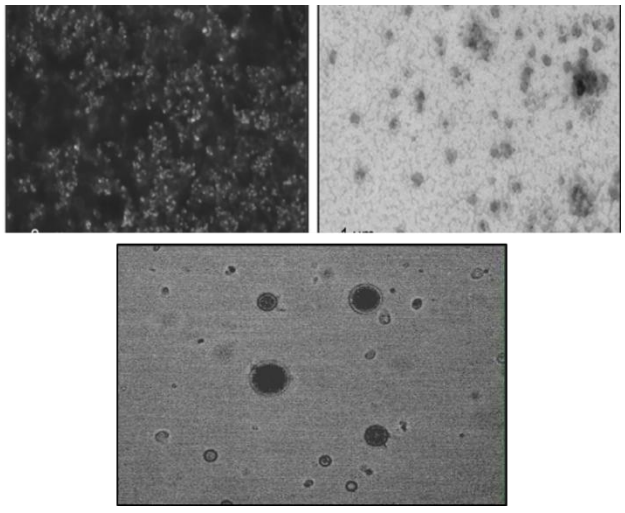

Figure. 10: The structurally morphology of DS by Motic image plus 1.8 ML software

The particles size distribution via intensity represented in the given **Fig. 11**as below:

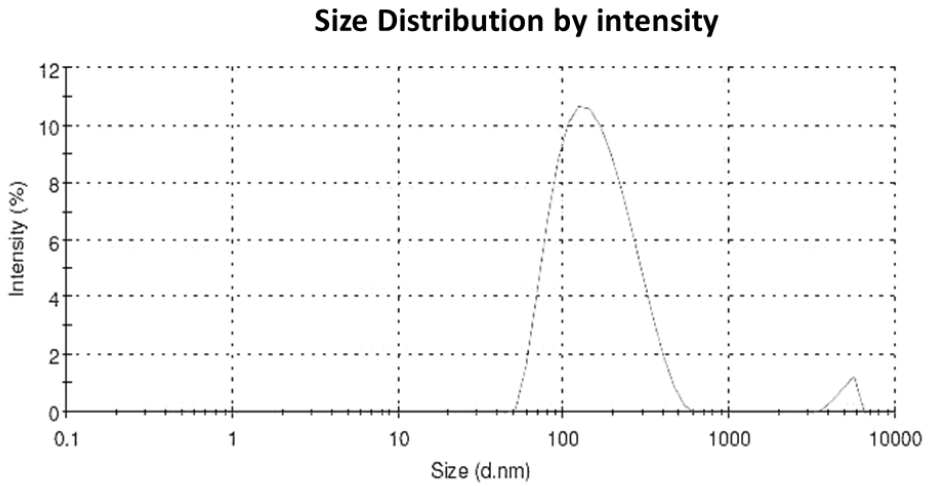

Figure. 11: The Particle size distribution via intensity of DS liposome formulation

The developed DS formulation was analyzed for particle size (**Fig. 11**) yielding measurements of 144.4 nm. The PDI was determined to be 0.224, indicating a restricted particle size distribution range. This low PDI suggests a high level of uniformity in the particle sizes.Additionally, it was observed that the behaviour, including sizedistribution and size, of the vesicles was entirely dependent on the selected variables. This finding was also reported in a previous study [39-40].

6. CONCLUSION

The current study utilized RSM, specifically a 3^2FFD, to successfully optimize DS formulations. The DSliposome was prepared using the TFH technique. The outcome of the optimization study indicated that a molar ratios of phospholipids and cholesterol (CH) at 2:1 demonstrated an increase in the extent and rate of In-Vitro drug release of DS from the design-optimized preparation. Therefore, it can be concluded that the suggested RSM approach may be beneficial for the optimization and preparation of liposomal preparation containing DS as NSAIDs.

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