FORMULATION AND CHARACTERIZATION OF *OROXYLUM INDICUM* LOADED PHYTOSOMESUSING BOX-BEHNKEN DESIGN

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Abstract

Phytosomes are a novel type of drug delivery system prepared by the incorporation of an active drug into the phospholipid vesicles. The present study was conducted to formulate and characterize phytosomes of *Oroxylum indicum*, an important medicinal plant, using Box-Behnken design. The independent variables studied were the concentrations of phospholipids cholesterol and dichloromethane, while the dependent variables were the particle size and entrapment efficiency of the phytosomes. The optimized formulation of Phytosomes OPF2 of *Oroxylum indicum* has been shown to be an effective drug delivery system, as it has been found to effectively improve the bioavailability of this medicinal plant extract. This is due to the unique physical structure of Phytosomes. The results of this study indicated that the Phytosomal formulation was more effective in enhancing the solubility and dissolution rate of *Oroxylum indicum* compared to the drug alone. This suggests that the Phytosomes of *Oroxylum indicum* could be a promising delivery system for this plant extract. The results of the study suggest that Box- Behnken design is a suitable approach for optimizing the formulation of phytosomes of *Oroxylum indicum* and other active drugs.

Keywords:*Oroxylum indicum, Phytosomes,* Box-Behnken design, Formulation, Characterization.

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Introduction

Phytosomes are a novel class of drug delivery systems that combine the advantages of both liposomes and plant extracts. Phytosomes are made up of a plant extract combined with phospholipids, which act as surfactants. They are capable of delivering drugs to the target site and enhancing their bioavailability and efficacy. Phytosomes are a type of formulation that contain natural phospholipids, such as phosphatidylcholine (PC) found in soy lecithin. PC is a component of cell membranes and is biodegradable. Studies have reported that phytosomes have hepatoprotective activity and improved pharmacokinetic and pharmacological parameters [1-3]. *Oroxylum indicum* (Linn), commonly known as the Indian trumpet flower, is a medicinal plant belonging to the family Bignoniaceae [4-5]. *O. indicum* belongs to Bignoniaceae family, widely found in Tropical Asia including Thailand. The chemical composition of *O. indicum* includes baicalein, chrysin, oroxylin A and oroxylin B [6 -7]. Many previous studies have reported

antioxidant [8], anti-inflammatory [9], anti-diabetic [10] and hepatoprotective properties for *O. indicum* and its is isolated compounds [11]. The purpose of this study is to formulate and characterize phytosomes of *Oroxylum indicum*

using Box-Behnken design.

Box-Behnken design is a type of response surface methodology, which is used to understand the relationships between the independent and dependent variables and to optimize the experimental design. The parameters studied were the amount of phospholipids, cholesterol and the concentration of dichloromethane. The phytosomes were characterized for particle size and entrapment efficiency. The study concluded that phytosomes of *Oroxylum indicum* can be successfully formulated using Box-Behnken design and can be used as a novel drug delivery system

Material and Methods

Formulation Development

The Box-Behnken design is a response surface methodology used to study the effect of different factors on a response. The design is used to optimize a process or product by finding the optimum factor levels for the desired response. In the development of phytosomes of *Oroxylum indicum*, the Box-Behnken design can be used to evaluate the effect of different factors on the response, such as the concentration of phospholipids, cholesterol and dichloromethane [12]. The design can be used to optimize the process parameters to obtain optimal results. The response can be measured in terms of the particle size and entrapment efficiency produced. The amounts of extract remain constant.

Preparation of phytosomes

This study was to optimize the phytosomal formulation of *Oroxylum indicum* using the Box-Behnken Design (BBD) of Design-Expert software. The formulation was developed using the BBD with a three-level, 2-factor design. The three factors were the type of concentrations of Phospholipids, Cholesterol and Dichloromethane, the amount of *Oroxylum indicum* extract remain constant.

The complex was prepared with phospholipids: Cholesterol and extract of aerial parts of *Oroxylum indicum* in the different ratio [13]. Weight amount of extract and phospholipids and cholesterol, were placed in a 100ml round- bottom flask and dichloromethane was added as reaction mediumas per table 1. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear mixture was evaporated and 20 ml of n-hexane was added to it with stirring. The precipitated was filtered and dried under vacuum to remove the

traces amount of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber colored glass bottle.

Evaluation of Prepared Phytosomes

Entrapment efficiency

Phytosome preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 4ºC[14].

The clear supernatant was siphoned off carefully to separate the non entrappedflavonoids and the absorbance of supernatant for non entrapped*Oroxylum indicum* was recorded at λ_{max} 420.0 nm using UV/visible spectrophotometer (Labindia 3000+). Amount of flavonoids in supernatant and sediment gave a total amount of *Oroxylum indicum* in 1 ml dispersion. The percent entrapment was calculated by following formula:

Particle size and size distribution

The particle size of optimized phytosomes formulations were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK) [15]. The electric potential of the phytosomes, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell.

Zeta potential analysis

The Zeta potentialof Phytosomes was measured using dynamic light scattering, Malvern zetasizer (Malvern zetasizer, Worcestershire, UK) [16]. Formulation was diluted with double distilled water and vortex for 5 minutes and then placed in the cell of the zeta sizer for analyze particle size of Phytosomes.

***In vitro* drug release study**

In vitro drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type) [17]. The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.5^{0}C and 75 rpm.10 mg of prepared phytosomes was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 12 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (37^{0}C) was replaced every time with the same quantity of the sample and takes the absorbance using spectroscopy.

Results and Discussion

The Box-Behnken design is a popular method for optimizing the formulation of phytosomal formulations. It involves varying the levels of three independent variables (concentrations of Phospholipids, Cholesterol and Dichloromethane) while keeping the levels of other components constant. The response is measured in terms of Entrapment efficiency and particle size. With the help of Design Expert software total 17 Formulations were prepared using varying amount of Phospholipids, Cholesterol and Dichloromethane (Table 1) and evaluated for Entrapment efficiency and particle size. Once the optimal formulation has been determined, the design expert software can be used to generate a graph that shows the relationship between the independent variables and the dependent variable. This graph can be used to identify the most important factors that affect the formulation of *Oroxylum indicum*. It can also be used to identify any trends in the data that could be used to further optimize the formulation. Finally, the results from the Box-Behnken design can be used to inform the development of a new formulation that can be optimized for Entrapment efficiency and particle

Table 1: Optimization of Phytosomal Formulation

F. Code	Std	Run	Factor 1: Phospholipids %	Factor 2: Cholesterol %	Factor 3: Dichloromethane (ml)
F1	10	1	1.25	1.5	10
F2	4	2	2	1.5	20
F3	1	3	0.5	0.5	20
F4	11	4	1.25	0.5	30
F5	16	5	1.25	1	20
F6	14	6	1.25	0.75	20
F7	7	7	0.5	1	30
F8	9	8	1.25	0.5	10
F9	6	9	2	1	10
F10	8	10	2	0.75	30
F11	12	11	1.25	1.5	30
F12	17	12	1.25	0.75	20
F13	3	13	0.5	1.5	20
F14	13	14	1.25	1	20
F15	5	15	0.5	1	10
F16	15	16	1.25	0.75	20
F17	2	17	2	0.5	20

Final Equation in Terms of Coded Factors

Entrapment efficiency =+64.72+3.49 A+1.91 B+1.80 C+0.1690 AB-2.83 AC-1.13 BC+4.28 A^2-2.01 B^2+0.0567 C^2

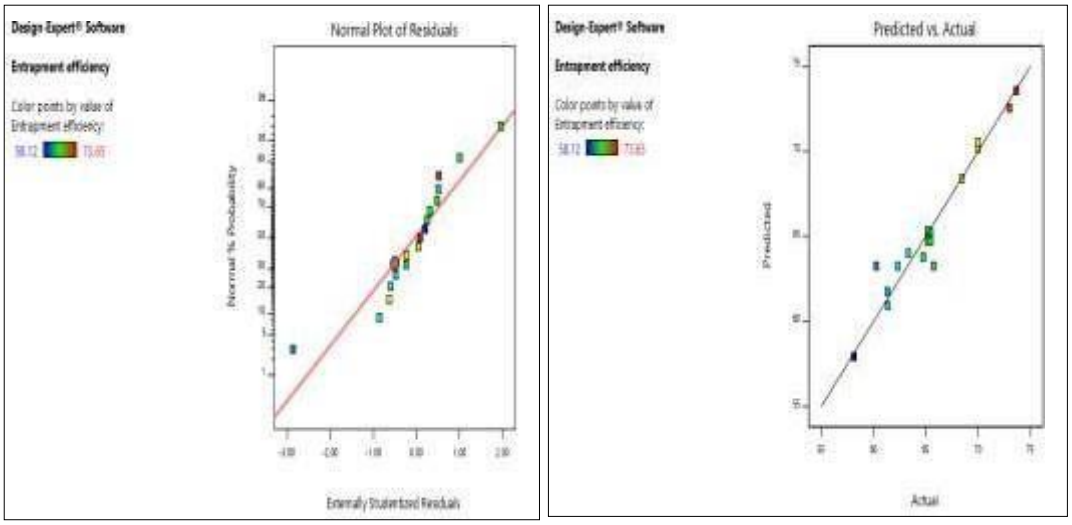
Final Equation in Terms of Actual Factors

Entrapment efficiency =+42.18172-7.26048 Phospholipids+23.82644 Cholesterol+0.854562 Dichloromethane+0.450656 Phospholipids * Cholesterol-0.377168 Phospholipids * Dichloromethane-0.226201 Cholesterol * Dichloromethane+7.60294 $Phospholipids^2$-8.02396 $Cholesterol^2$+0.000567 Dichloromethane ^{2}

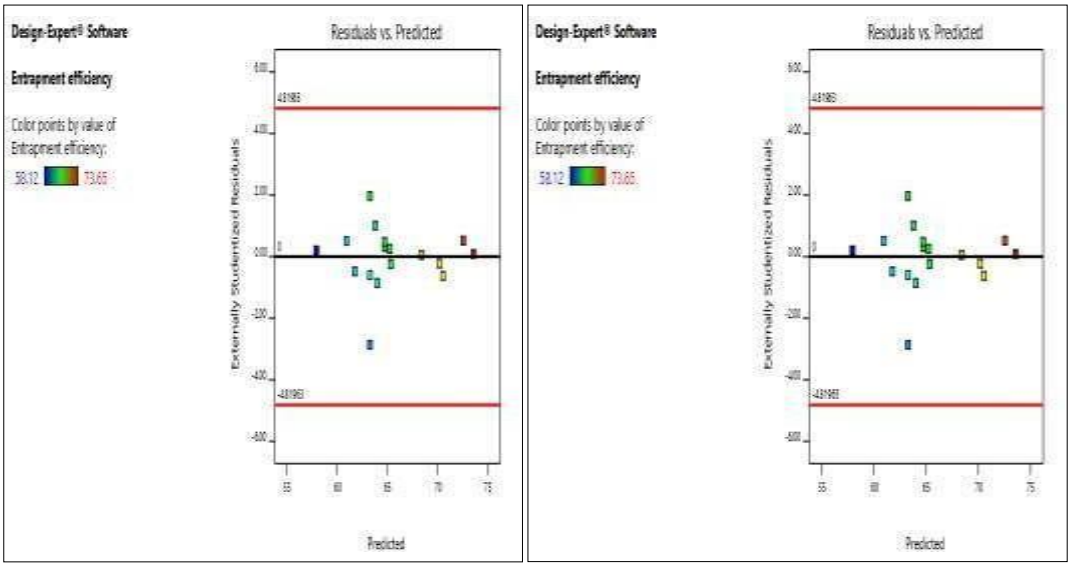
Final Equation in Terms of Coded Factors

Particle size =+128.92-6.31A-12.16B+6.15C+2.88AB+17.39 AC+20.66 BC+5.07 A^2+13.82 B^2+15.58 C^2

Particle size =+433.34779-85.02168 Phospholipids-227.13735 Cholesterol-12.64568 Dichloromethane+7.69179 Phospholipids * Cholesterol+2.31835 Phospholipids * Dichloromethane+4.13238 Cholesterol * Dichloromethane+9.02055 $Phospholipids^2$+55.28214 $Cholesterol^2$+0.155766 Dichloromethane ^{2}

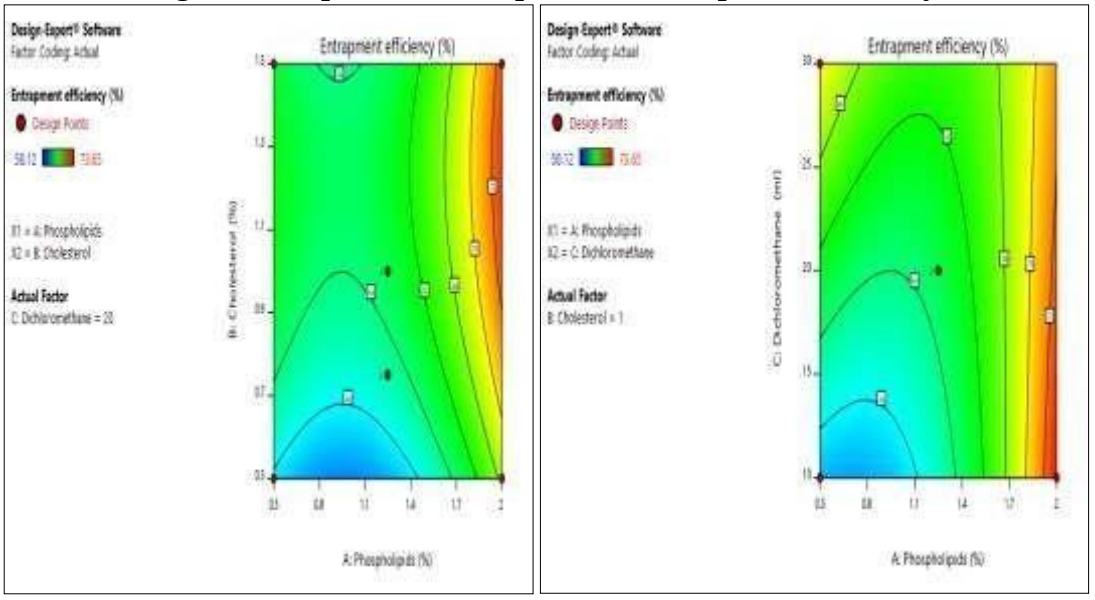


Normal Plot of ResidualsPredicted vs. Actual

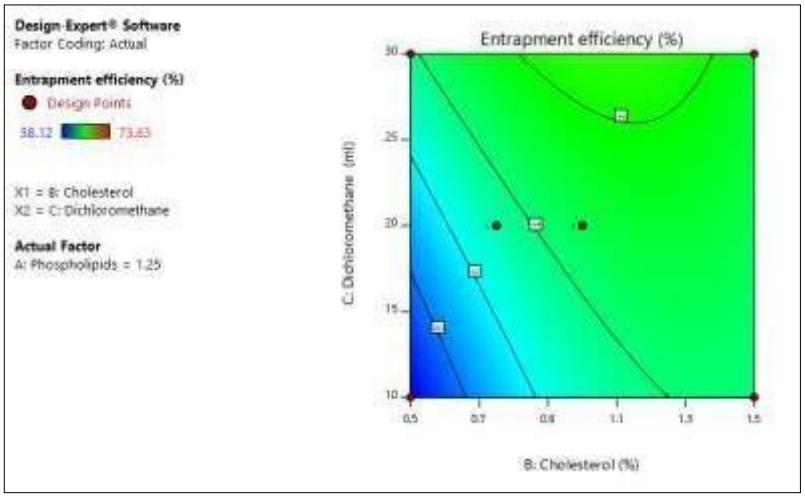


Predicted vs. Actual **Residuals vs. Predicted**

Figure 1:Response surface plots for Entrapment efficiency

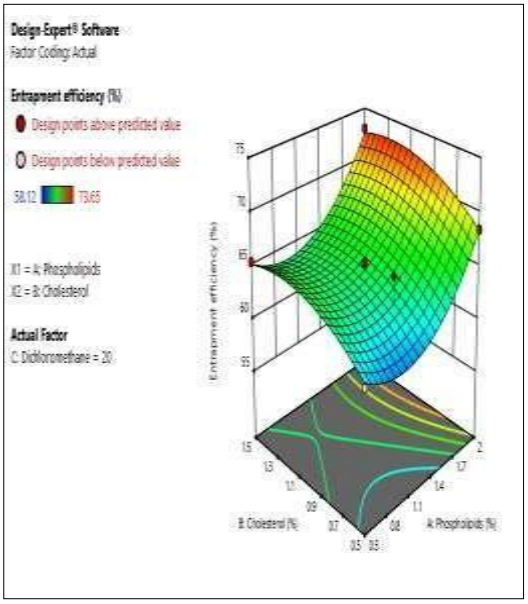


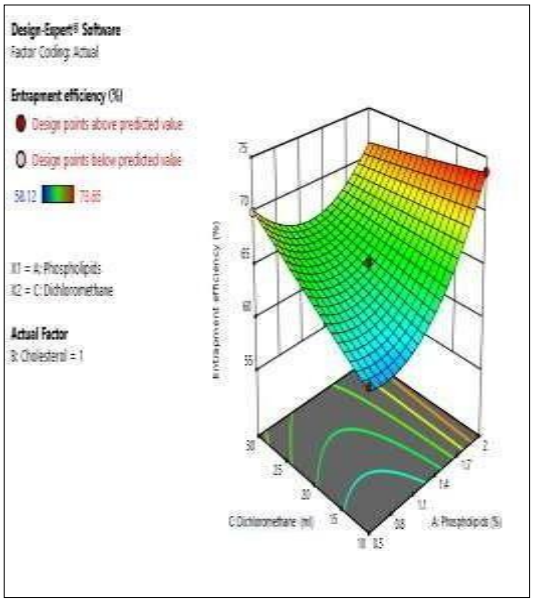
Between Phospholipid and cholesterol **Between Phospholipid and Dichloromethane**



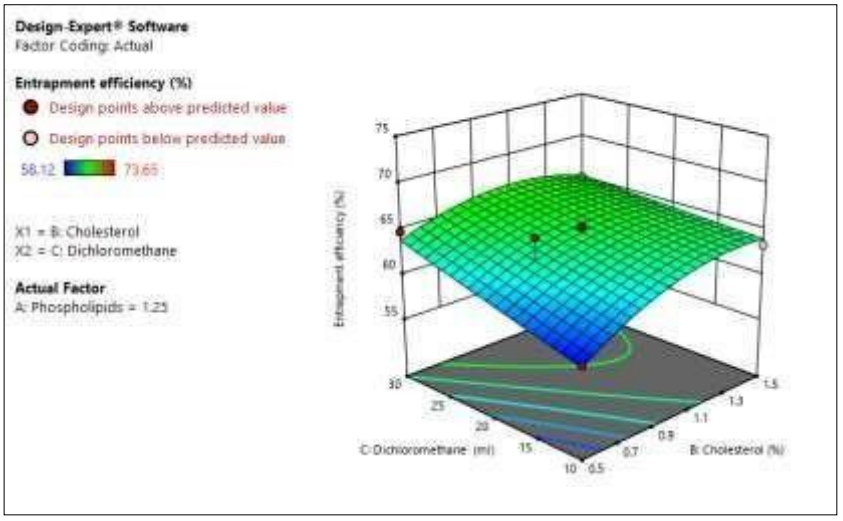
Between Cholesterol and Dichloromethane

Figure 2: Contourplots for Entrapment efficiency

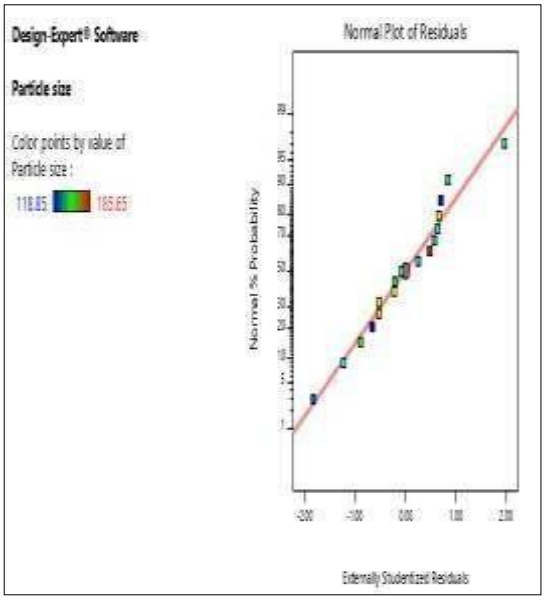


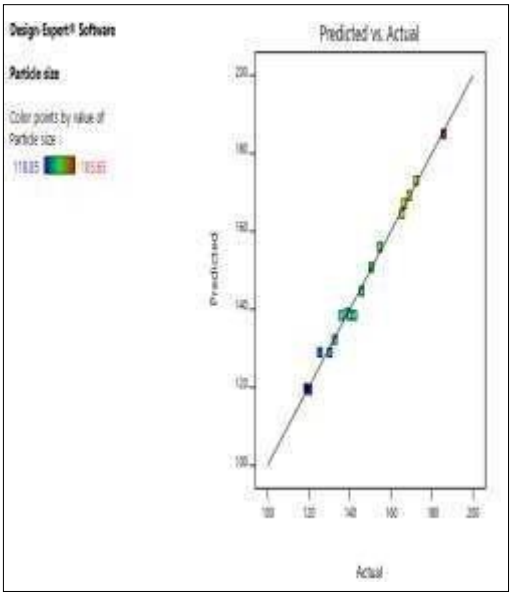


Between Phospholipids and Cholesterol **Between Phospholipids and Dichloromethane**

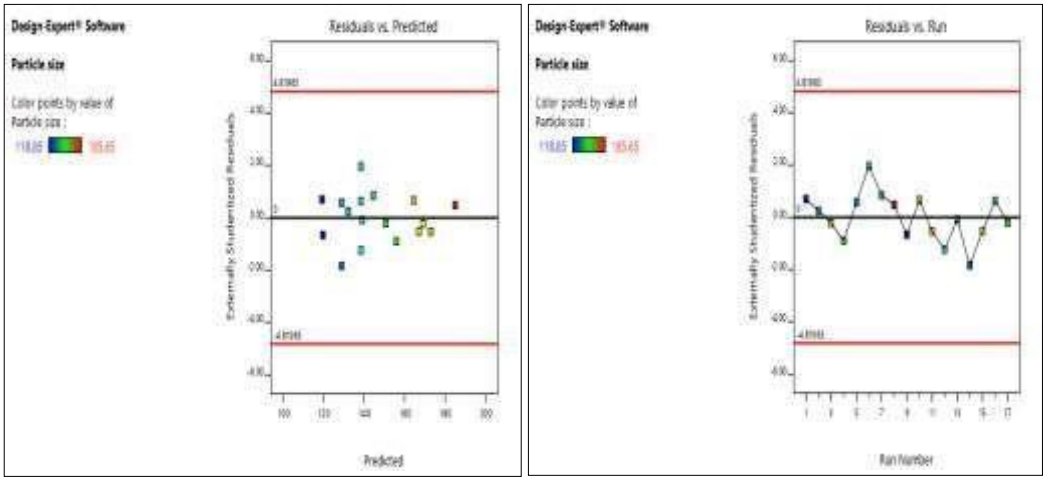


Between Cholesterol and Dichloromethane
Figure 3:3D surface plots for Entrapment efficiency





Normal Plot of Residuals **Predicted vs. Actual**



Residuals vs. Predicted **Residuals vs. Run**

Figure 4: Response surface plots for Particle Size

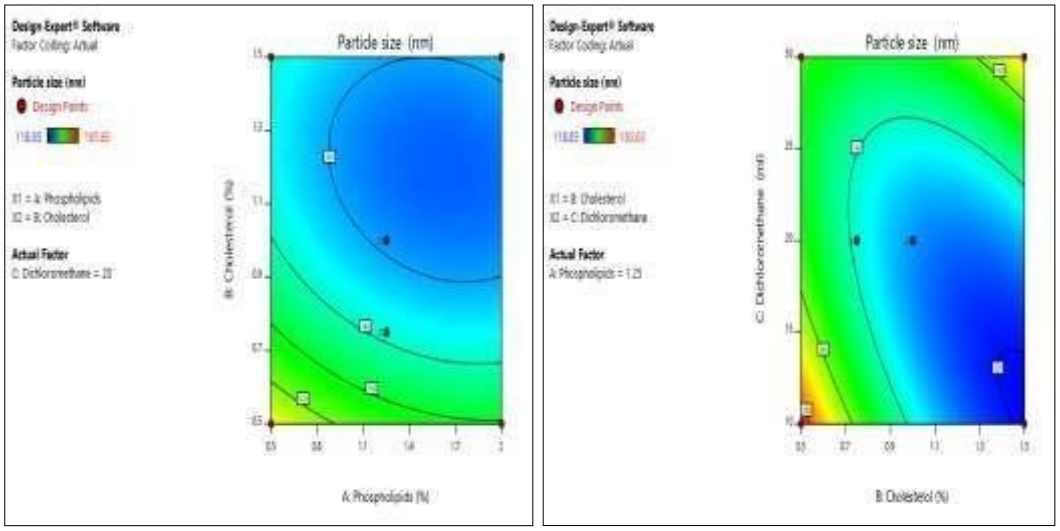
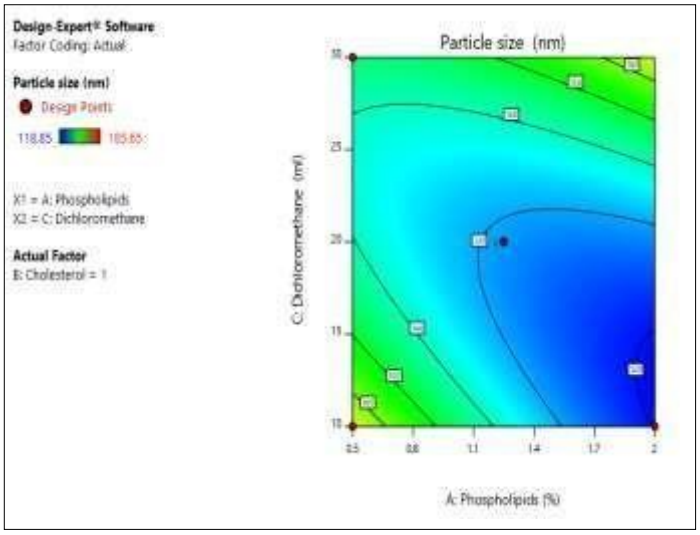
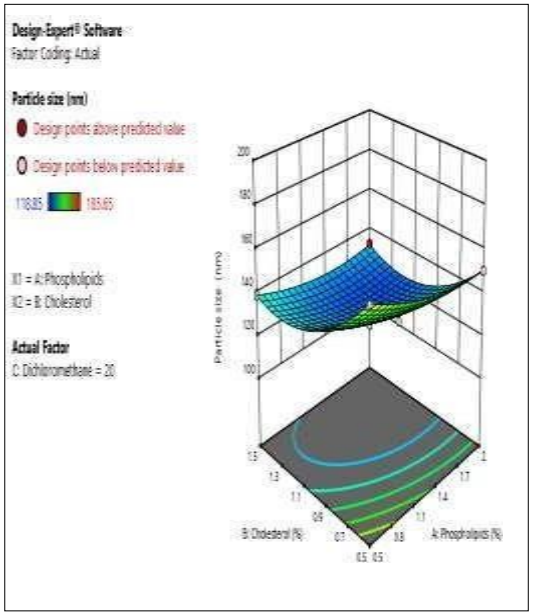


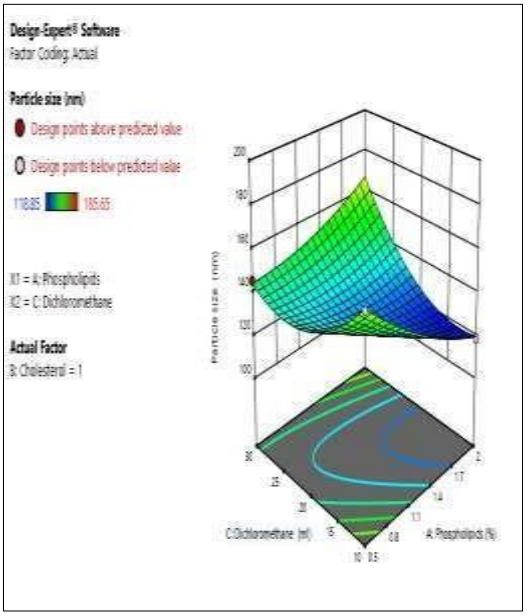
Figure 5: Contourplots for Particle Size (Between Phospholipid and cholesterol)



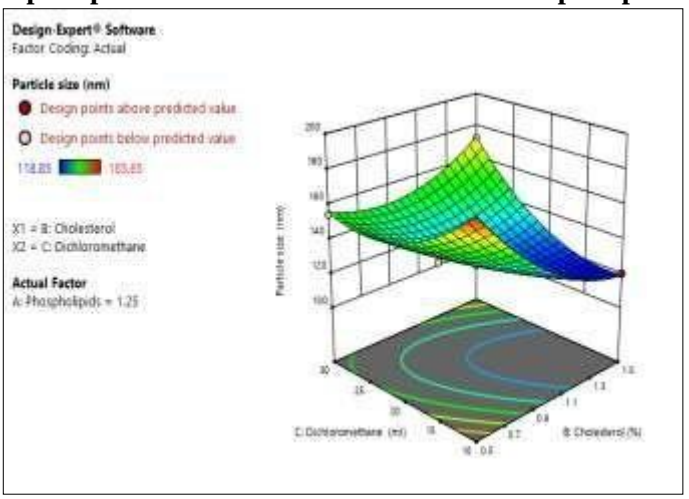
Between Phospholipid and Dichloromethane

Figure 6: Contourplots for Particle Size





Between Phospholipids and Cholesterol **Between Phospholipids and Dichloromethane**



Between Cholesterol and Dichloromethane

Figure 7:3D surface plots for Particle Size

Results of Particle size and Entrapment efficiency

The results of the particle size and entrapment efficiency of phytosomes showed that the particle size of the phytosomes was found to be between 118.85-185.65 nanometers and the entrapment efficiency was found to be around 58.12-73.65%. This indicates that phytosomes are suitable for drug delivery applications as they are able to transport the drug to the desired target and also have high entrapment efficiency. Additionally, the small particle size of the phytosomes allows for better absorption and faster release of the drug into the body.

Table 2: Results of Particle size and Entrapment efficiency

F. Code	Entrapment efficiency (%)	Particle size (nm)
F1	63.32	120.23
F2	72.98	132.56
F3	61.32	168.85
F4	64.74	154.65
F5	65.45	130.25
F6	60.25	142.23
F7	69.98	145.74
F8	58.12	185.65
F9	73.65	118.85
F10	69.98	165.45
F11	65.14	172.32
F12	62.32	135.74
F13	65.45	138.98
F14	65.20	125.45
F15	61.36	166.45
F16	65.74	139.98
F17	68.45	150.45

Experimental results with predicted responses:

The experimental results with predicted responses can be used to measure the accuracy of the predictions. This can be done by comparing the actual responses of the participants in the experiment to the predicted responses. If the predicted responses are accurate, then the results of the experiment should closely match the predicted responses. If the predicted responses are not accurate, then the results of the experiment should not match the predicted responses. The accuracy of the predictions can also be measured by considering the accuracy of the predicted response rate, or the number of correct responses out of the total number of responses. On the basis of DOE two formulations (Run order 9 & 14) is selected as optimized formulation for preparation of Phytosomes because the results of experimental values for composition of Phytosomes are more similar to the predicted values.

Table 3: Experimental results with predicted responses

Formulation	Run Order	Composition (%) Phospholipids/Cholesterol/ Dichloromethane	Response	Actual Value	Predicted value
OPF1	9	2/1/10	Particle Size	118.85	119.73
			Entrapment Efficiency	73.65	73.57
OPF2	14	1.25/1/20	Particle Size	125.45	128.92
			Entrapment Efficiency	65.20	64.72

Table 4: Results of zeta potential of optimized phytosomes formulations

S. No.	Formulation Code	Zeta potential (mV)
1	OPF1	-36.85
2	OPF2	-41.74

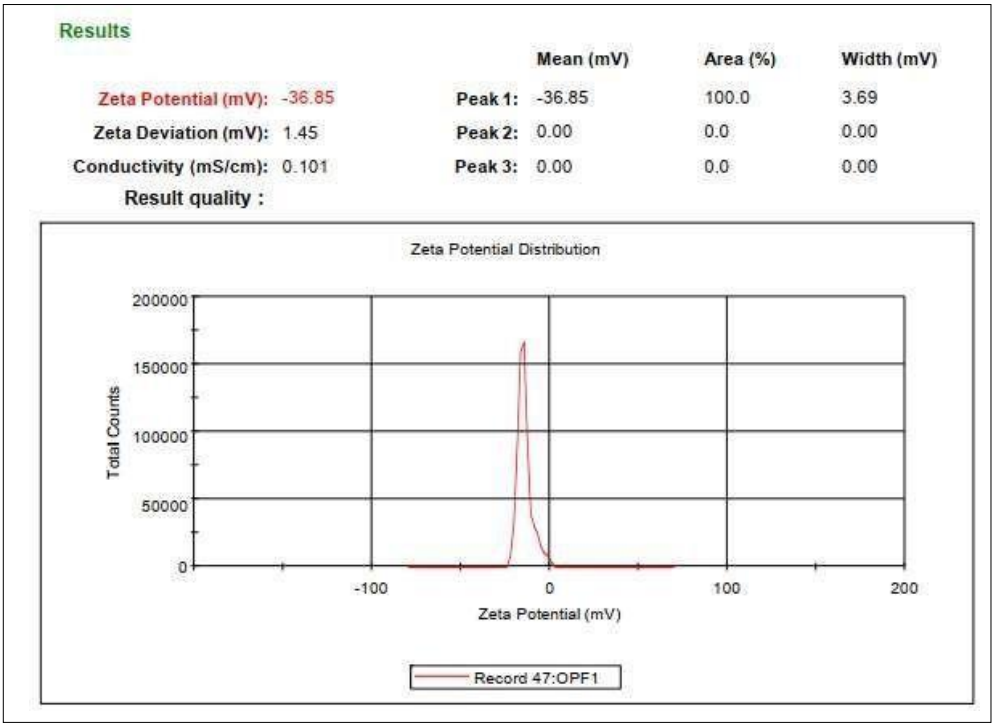


Figure 8: Zeta potential of optimized phytosomes formulations OPF1

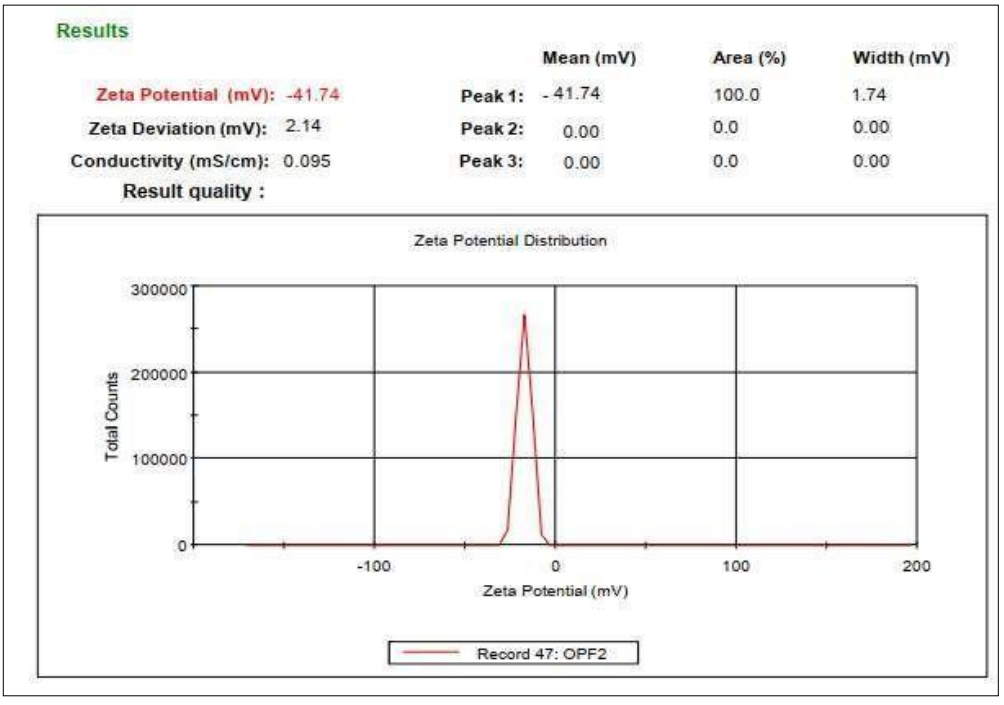


Figure 9: Zeta potential of optimized phytosomes formulations OPF2

Drug release from optimized formulations

The optimized formulation of Phytosomes OPF2 of *Oroxylum indicum* has been shown to be an effective drug delivery system, as it has been found to effectively improve the bioavailability of this medicinal plant extract. This is due to the unique physical structure of Phytosomes, This allows for the drug to be encapsulated within the Phospholipid complex and released in a controlled manner. The optimized formulation of Phytosomes of *Oroxylum indicum* has been shown to be effective in improving the bioavailability of the medicinal plant extract. The Optimized formulation OPF2 showed the release from Phospholipids complex in sustain manner.

Overall, the optimized formulation of Phytosomes of *Oroxylum indicum* is an effective drug delivery system. It has been found to effectively improve the bioavailability of the medicinal plant extract, while also providing sustained-release properties. This makes it an ideal option for the treatment of various diseases.

Table 5: Drug release from optimized formulations

S.No.	Time (hr)	Extract	% Cumulative drug release from Phytosomesformulation	
			OPF1	OPF2
1.	0	0	0	0
2.	0.5	26.65	23.32	18.85
3.	1	45.85	35.65	26.65
4.	2	69.98	49.98	38.85
5.	3	86.65	58.85	45.65
6.	4	92.23	69.98	55.85
7.	6	-	95.65	69.98
8.	8	-	98.85	73.32
9.	10	-	99.05	86.65
10.	12	-	99.65	99.12

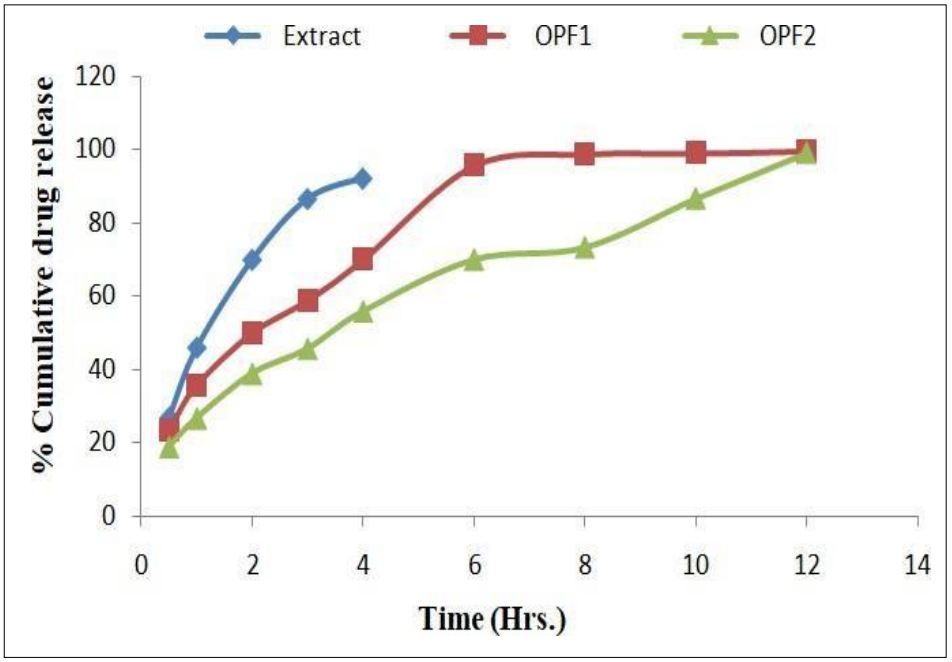


Figure 8: *In vitro* drug release from optimized formulation

Conclusion

The box-behnken design study concluded that the Phytosome formulation of *Oroxylum indicum* showed a significant improvement in the dissolution rate of the drug from the Phytosomes compared to the drug alone. The results of this study indicated that the Phytosomal formulation was more effective in enhancing the solubility and dissolution rate of *Oroxylum indicum* compared to the drug alone. This suggests that the Phytosomes of *Oroxylum indicum* could be a promising delivery system for this plant extract.

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