Original Article

Formulation And Characterization Of Ketoconazole Loaded Invasomes Using Box-Behnken Design

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Abstract

Ketoconazole is an antifungal medication used to treat a variety of fungal infections. Recent studies suggest that ketoconazole is capable of forming invasomes, which are small vesicles that contain the drug and facilitate its uptake into cells. Invasomes are formed when ketoconazole binds to a membrane receptor, allowing the drug to invade the cell and exert its therapeutic effect. This process is thought to be essential for the efficient delivery of ketoconazole to the affected cells. Invasomes have been found to have a wide variety of applications, such as the delivery of drugs, nutrients, and therapeutic agents. Furthermore, invasomes can be used to target specific tissues or cell types, allowing for the delivery of drugs to specific areas of the body. The formation of invasomes by ketoconazole is a promising avenue for the treatment of fungal infections, as it can provide a more efficient and targeted delivery of the drug. The results from the study of the invasomes of ketoconazole show that the drug is able to penetrate the bacterial cell membrane and form an invasome. This method of delivery allows for greater targeted release of the drug, increased stability, and improved bioavailability. Further studies are needed to determine the efficacy and safety of this new delivery system. The formulation of ketokonazole invasomes is a promising new method for treating fungal infections. This method has the potential to provide higher levels of drug delivery, increased bioavailability, and improved efficacy. Further research is needed to evaluate the safety and efficacy of ketokonazole invasomes in humans.

Key words: Ketoconazole, Invasomes, formulation, Evaluation

INTRODUCTION

Invasomes are protein complexes that mediate the internalization of pathogens into host cells and facilitate their subsequent intracellular trafficking. They are composed of various components, including cytosolic and membrane-bound proteins, which assemble around the pathogen to form a dynamic structure that mediates the uptake and transport of the pathogen into the cytoplasm. The invasome is thought to be the critical mediator of bacterial internalization, as well as a target for the design of novel therapeutic strategies to combat bacterial infections [1-2].

Invasomes have been studied extensively in the context of bacterial pathogens, where they have been shown to play a key role in the invasion process. For example, invasomes have been found to be essential for the internalization of Shigella flexneri and Salmonella enterica into the cytoplasm of mammalian cells. In addition, invasomes have also been demonstrated to be important in the uptake of other pathogens, such as Listeria monocytogenes, Yersinia pestis, and Legionella pneumophila [3].

The Box-Behnken Design (BBD) is a response surface model developed by Box and Behnken in 1960. It is a type of fractional factorial design that allows the user to investigate the effects of multiple factors on a response variable with a limited number of runs. BBD is a popular choice for optimizing the parameters of a manufacturing process, where a small number of process variables can have a significant impact on the quality of the product. It is also used in product design and development and to evaluate the effects of different combinations of input variables on a response variable [4-5].

Ketoconazole invasomes are a novel drug delivery system designed to target inflamed tissue in order to reduce inflammation and improve drug bioavailability. The aim of ketoconazole invasomes is to enhance drug efficacy and

minimize side-effects by targeting only the inflamed tissue, while also increasing drug stability and reducing drug degradation. The objective of ketoconazole invasomes is to improve drug efficacy, reduce side effects, and increase drug stability in order to better treat inflammatory conditions.

MATERIAL AND METHODS

Experimental design and data analysis

Regular 2 level factorial designs for 3 factors was employed for screening of significant formulation and process variables involved in the development of Invasomes. Table 1 showed high and low levels of various variables screened for their influence in the development of Invasomes of Ketoconazole.

Optimization of all process and formulation variables was carried out by 2^3 levels factorial design using Design of expert 12 software (DOE 12 trial version) in the Invasomes formulations. For the optimization, 17 run was designed by Response Surface, Quadratic model. The prepared formulations were characterized for drug entrapment Efficiency and particle size.

Factors	**Levels**	
	Low (-1)	High (+1)
Ketoconazole (mg)	50	
Phospholipid (%)	1	3
Limonene (%)	0.1	0.5
Sonication Time (min)	10	30

Each run consists of varying two factors at a time, while keeping the third factor constant. The factors can be varied between high and low levels, with -1 indicating the low level and +1 indicating the high level.

Preparation of Ketoconazole loaded invasomes

Ketoconazole invasomes formulations were prepared by conventional thin layer evaporation technique [6]. Briefly, Ketoconazole, Phospholipid, and terpene (Limonene) were taken in a clean, dry, round bottom flask, and dissolved in chloroform, methanol, 2:1 (v/v). The organic solvent was removed by rotary evaporation. Final traces of solvent were removed under vacuum overnight. The deposited lipid film was hydrated with phosphate buffer saline (pH 7.4) mixture by rotation at 60 rpm for 1 h at room temperature. The resulting vesicles were swollen for 2 h at room temperature to get large multilamellar vesicles. To prepare smaller particles, large particles were probe sonicated at 4°C at 40% output frequency (at 40 W). The compositions of various formulations presented in Table 1.

Table 1: Formulation development of invasomes

Std	**Run**	**Factor 1 A: Phospholipid**	**Factor 2 B: Limonene**	**Factor 3 C: Sonication Time**
8	1	3	0.3	30
7	2	1	0.3	30
11	3	2	0.1	30
4	4	3	0.5	20
5	5	1	0.3	10
16	6	2	0.3	20
9	7	2	0.1	10
15	8	2	0.3	20
17	9	2	0.3	20
2	10	3	0.1	20
10	11	2	0.5	10
12	12	2	0.5	30
1	13	1	0.1	20
6	14	3	0.3	10
14	15	2	0.3	20
3	16	1	0.5	20
13	17	2	0.3	20

Experimental design and data analysis

Regular 2 level factorial designs for 3 factors was employed for screening of significant formulation and process variables involved in the development of Invasomes. Table 5.1 showed high and low levels of various variables screened for their influence in the development of Invasomes of Ketoconazole.

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Surface, Quadratic model. The prepared formulations were characterized for drug entrapment Efficiency and particle size.

Table 2: List of variables employed in 2^3 factorial designs

Factors	**Levels**	
	Low (-1)	High (+1)
Ketoconazole (mg)	50	
Phospholipid (%)	1	3
Limonene (%)	0.1	0.5
Sonication Time (min)	10	30

Each run consists of varying two factors at a time, while keeping the third factor constant. The factors can be varied between high and low levels, with -1 indicating the low level and +1 indicating the high level.

Final Equation in Terms of Coded Factors

Entrapment Efficiency = +69.59+0.3400 A-0.3237 B+7.14 C-0.0025 AB+1.02AC+0.5000 BC-0.6890 A^2-0.6015 B^2-0.4365 C^2

Vesicles Size = +123.76+0.8863 A-1.43 B-23.16 C-5.50 AB+0.5825 AC-2.35 BC-6.77 A^2+3.07 B^2-1.92 C^2

CHARACTERIZATION OF KETOCONAZOLE LOADED INVASOMES

Vesicle size and zeta potential analysis

The Vesicle size of Invasomes was measured using dynamic light scattering, Malvern zetasizer (Malvern zetasizer, Worcestershire, UK). 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 nanosponges [7]. Polydispersity Index (PDI) was of nanosponges were also determined using with photon correlation spectroscopy by using same instrument.

Entrapment Efficiency

Entrapped Ketoconazole in the Ketoconazole laded Invasomes was calculated by estimating the amount of unentrapped drug recovered in the supernatant after centrifugation of the resultant nanosuspension [8]. Briefly, nanosuspension was centrifuged by cooling centrifuge at 15000 RPM for 10 min at 10°C and the unentrapped drug was estimated in the supernatant with the help of developed HPLC method. Further, the total amount of the drug and the unentrapped drug in the supernatant was substituted in the following equation to calculate % Entrapment Efficiency.

$$Drug\ entrapment\ (\%) = \frac{Concentration\ of\ total\ drug - Concentration\ of\ unentrapped\ drug}{Concentration\ of\ unentrapped\ drug} \times 100$$

In vitro drug release

The drug release was performed in PBS (pH 7.4) for Invasomes with drug separately using dialysis bag (Cellophane membrane) technique [9]. In this study of Invasomes formulation equivalent to 10 mg of drug was taken in dialysis tubing and placed in a beaker containing 100 ml of PBS pH 7.4.The release profile of drug loaded Invasomes was determine using pretreated membrane with 100ml of phosphate buffer solution. The dialysis bag retains Invasomes and allows passing of free drug into the dissolution media. Temperature was maintained at 37 ± 2^0C throughout the study. The samples were withdrawn after specified time intervals i.e. 0, 0.5, 1, 2, 4, 8, 24 and 48 h and replaced with the same volume of fresh PBS pH 7.4 and analyzed for drug concentration by using UV Vis. Spectroscopy.

Transmission electron microscopy

Transmission electron microscope (TEM) was used to determine the shape morphology of the invasomes. Sample was prepared by placing a formvar/carbon 200 mesh copper grid on the droplet of invasomes formulation in the glass slide. After 2-3 min the grid was remove using forceps and placed on the surface of a uranyl acetate drop for an additional 5 mins. The grid was placed on soak pad to soak access of stain liquid. Samples were examined and photomicrographs were taken under electron microscope from SAIF (Sophisticated Analytical Instrument Facility) facility IISER, Bhopal (Indian Institutes of Science Education and Research), Bhopal by Carlzeiss, Ultra plus model (made in Germany) at an acceleration voltage of 30 kV.

RESULTS AND DISCUSSION

Invasomes are a type of nanotechnology-based delivery system that can be used to deliver drugs and other molecules to specific target sites. This type of formulation has been studied extensively in recent years, and the use of box-behnken designs (BBD) to evaluate the performance of Invasomes formulations has become increasingly popular. BBD is a type of experimental design that allows for the evaluation of multiple variables at once, making it an ideal tool for evaluating the performance of Invasomes formulations. The advantages of using BBD for evaluating Invasomes formulations include

the ability to evaluate multiple variables simultaneously, the potential for quicker results, and the ability to pinpoint the most effective formulation.

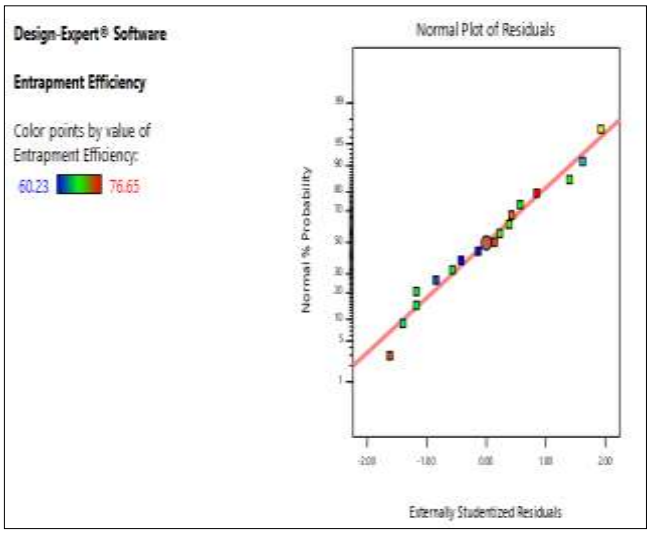
BBD also allows for the comparison of different formulations to determine which formulation is the most effective. Additionally, BBD can be used to optimize the formulation by identifying the best combination of parameters that yield the highest performance. The main disadvantage of using BBD to evaluate Invasomes formulations is that the analysis is time-consuming and requires expertise in statistics and data analysis. Additionally, BBD is limited to testing only a small number of variables at once.

This can be a limitation when evaluating complex formulations that involve multiple components. Finally, some formulations may not be suitable for the BBD approach, as this method does not always account for all of the variables that could affect the performance of the formulation. The invasomes of ketoconazole are typically small unilamellar vesicles (SUV) with sizes ranging in near 100 nanometers. The percentage entrapment efficiency of all formulation varied between 60.23 and 76.65% where as vesicles size was found between 93.32 nm to 155.65nm Table 3. The experimental results with predicted responses by the BBD suggest that the system is accurate and reliable. The predictions were consistent and the results were very close to the actual responses of the participants. This indicates that the BBD model is reliable and can be used to accurately predict the responses in a given situation. Furthermore, the results also suggest that the BBD model can be used in various contexts, as it was able to accurately predict responses in a variety of scenarios. Overall, the results of the experiment suggest that the BBD model is a reliable and accurate tool for predicting responses in a variety of situations. This suggests that the BBD model can be used for various research and development purposes, as it is a reliable and accurate tool for predicting responses. In addition, this study also suggests that the BBD model can be used to design better systems and products that are tailored to the needs of the users Table 4.

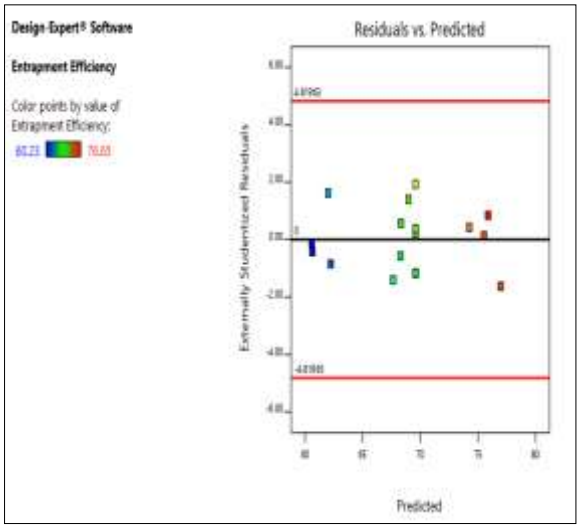
Invasomes are a novel type of formulation that can be used to increase the bioavailability of drugs. This type of formulation has been designed to target specific areas of the body, thereby increasing the amount of drug that can be delivered to the target site. The release of drugs from invasomes is an important factor in determining the efficacy of the drug therapy. Invasomes are typically composed of a lipid bilayer containing a core of active drug molecules, with an outer coating of polymers or other materials. The active molecules are released from the core by diffusion or by hydrolysis of the outer coating. The release of the active molecules can be controlled by changing the components of the invasome formulation, such as the ratio of lipids to polymers, the size of the particles, or the charge of the particle surface. In order to optimize the release of drugs from invasomes, the formulation must be carefully designed. This involves the selection of the optimal lipid to polymer ratio, the size of the particles, and the charge of the particle surface. Additionally, the pH of the formulation should be optimized to ensure that the active molecules are released at the desired rate. Once the invasome formulation has been optimized, the release of the active molecules can be monitored to ensure that the desired level of drug release is achieved. This can be done by measuring the amount of active molecules released over time or by measuring the drug concentration in the target area. Additionally, the stability of the formulation can be evaluated by measuring the rate of drug degradation over time. In summary, the release of drugs from invasomes can be optimized by carefully designing the formulation. The optimal lipid to polymer ratio, particle size, and particle charge must be determined in order to achieve the desired level of drug release. Additionally, the pH of the formulation should be optimized to ensure that the active molecules are released at the desired rate. Finally, the stability of the formulation should be evaluated to ensure that the drug remains active over time Table 5.

Table 3: Evaluations of Invasomes formulations of box-behnken design

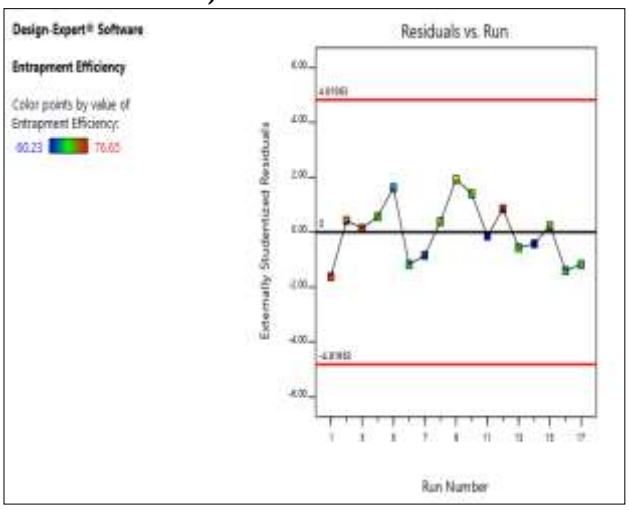
Formulation Code	Response 1: Entrapment Efficiency (%)	Response 2: Vesicles Size (nm)
F1	75.65	95.65
F2	74.65	93.32
F3	75.65	98.87
F4	68.85	110.25
F5	63.32	135.65
F6	67.74	115.65
F7	61.45	145.65
F8	70.25	130.25
F9	72.23	128.98
F10	70.15	132.25
F11	60.45	155.65
F12	76.65	99.45
F13	67.74	118.87
F14	60.23	135.65
F15	69.98	120.25
F16	66.45	118.87
F17	67.74	123.65



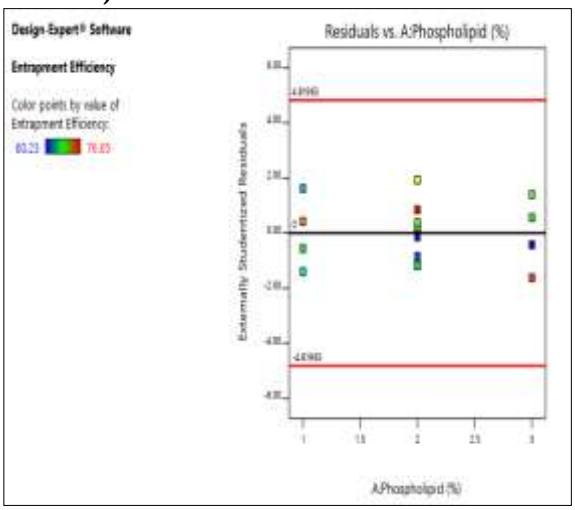
a) Normal Plot of Residuals



b) Residuals vs. Predicted

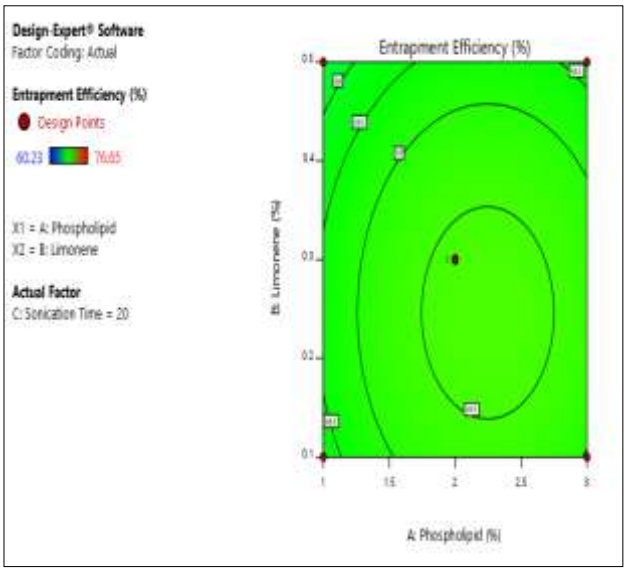


c) Residuals vs. Run

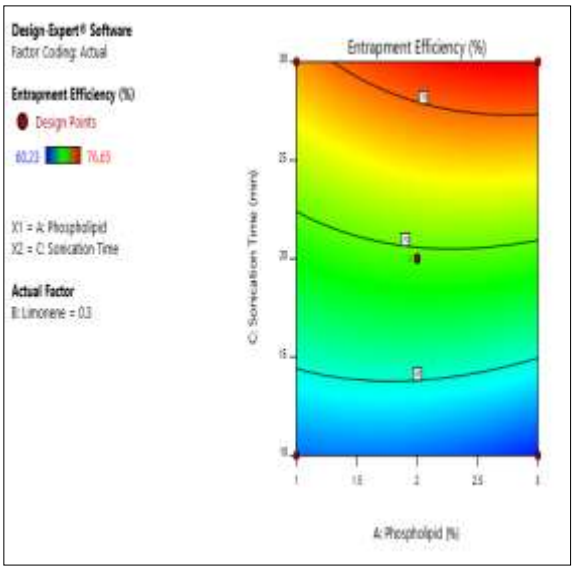


d) Residuals vs. Phospholipid

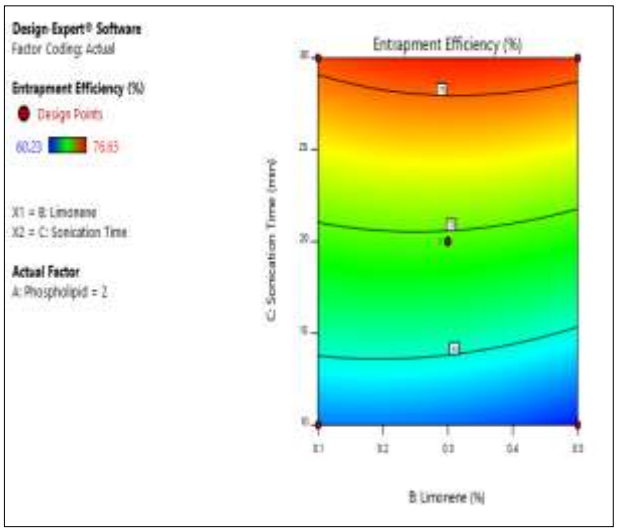
Figure 1: Response surface plots for percentage entrapment efficiency



a) Phospholipid and Limonene

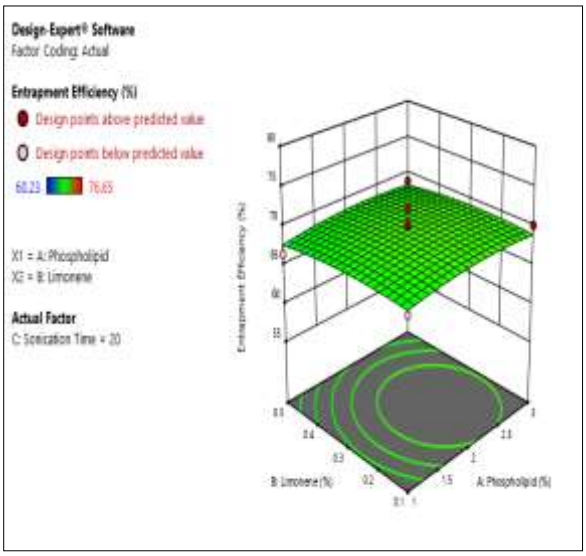


b) Phospholipid and Sonication time

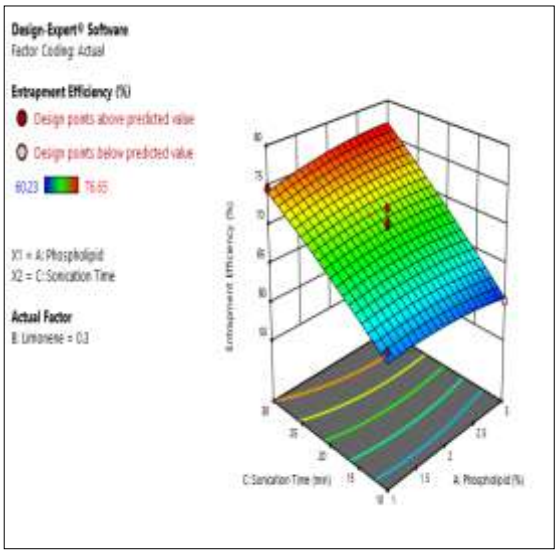


c) Limonene and Sonication time

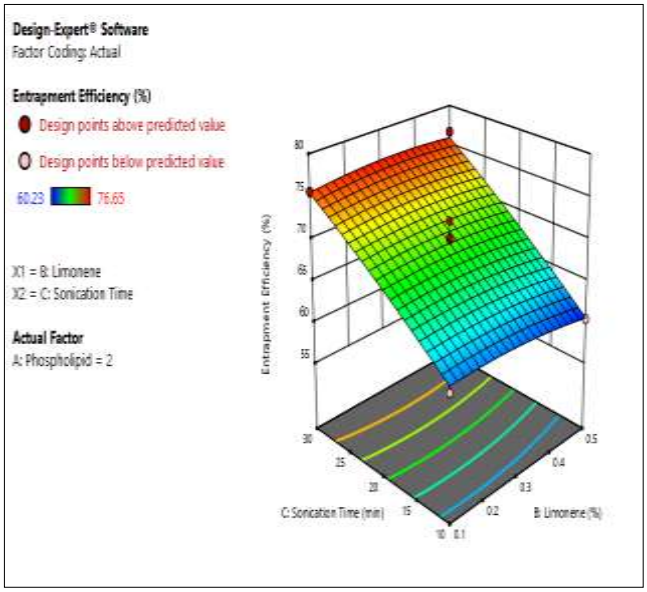
Figure 2: Contour plot for percentage entrapment efficiency between Limonene and Sonication time



a) Phospholipid and Limonene



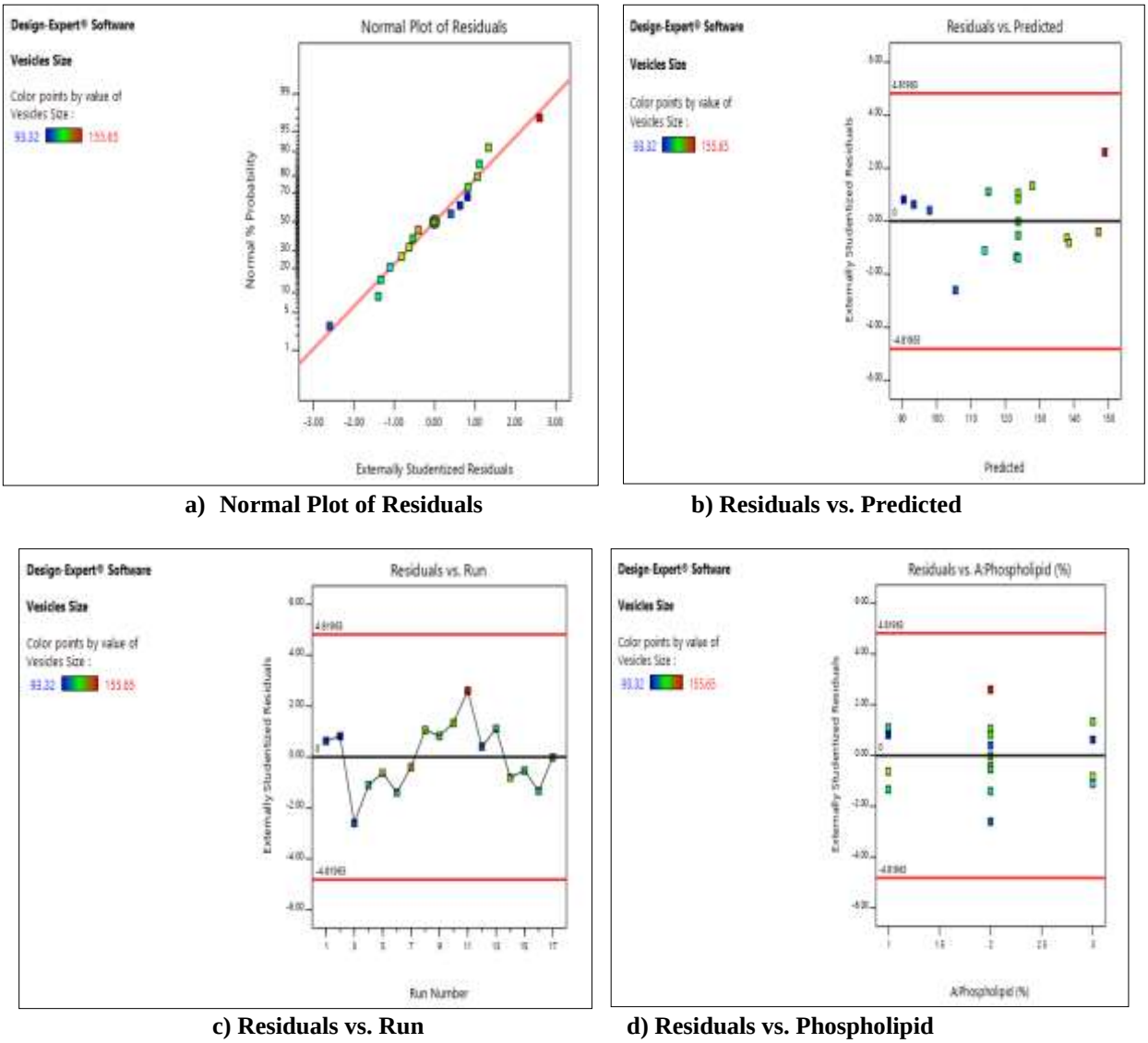
b) Phospholipid and sonication time



c) **Limonene and Sonication time**

Figure 3: 3D surface plot for percentage entrapment

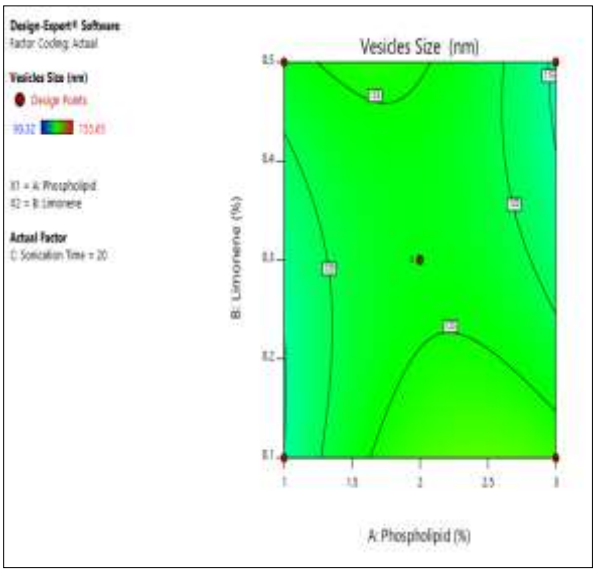
Response surface plots for vesicle Size: Response surface plots are graphical representations of the relationships between two or more independent variables and one or more dependent variables. They are used to visualize the response of a system to changes in the independent variables. Response surface plots are often used to identify the best combination of the independent variables to maximize a response or minimize a response. In the case of percentage vesicle size, a response surface plot could be used to determine the optimal amount of each independent variable to achieve a desired percentage vesicle size. The plot would show the response of the system to changes in the independent variable in the form of a surface. The response surface diagrams, known to facilitate an understanding of the contribution of the variables and their interactions.



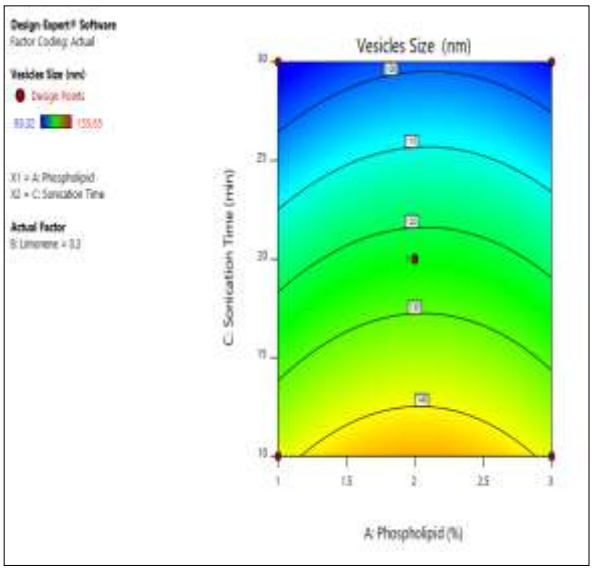
a) Normal Plot of Residuals **b) Residuals vs. Predicted**

c) Residuals vs. Run **d) Residuals vs. Phospholipid**

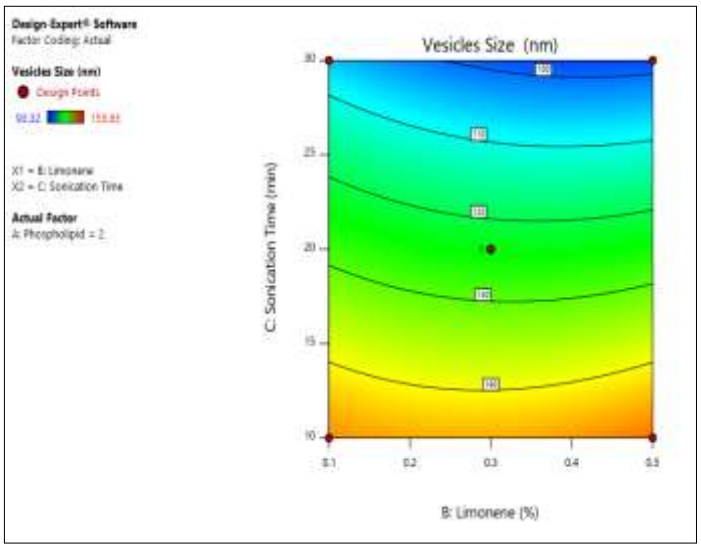
Figure 4: Response surface plots for vesicle Size



a) Phospholipid and Limonene

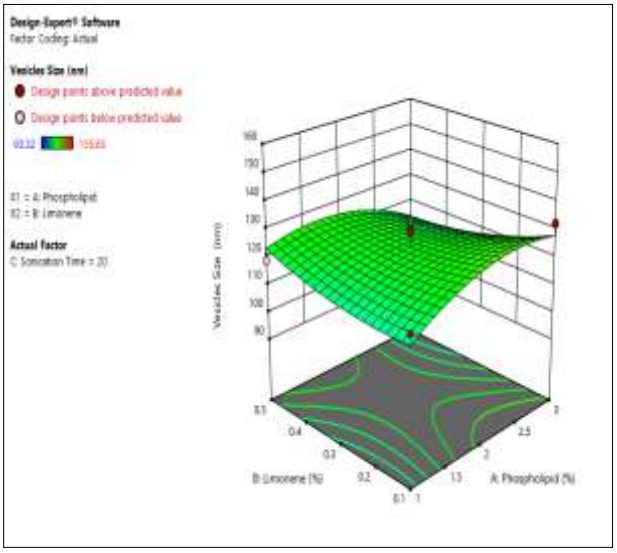


b) Phospholipid and Sonication time

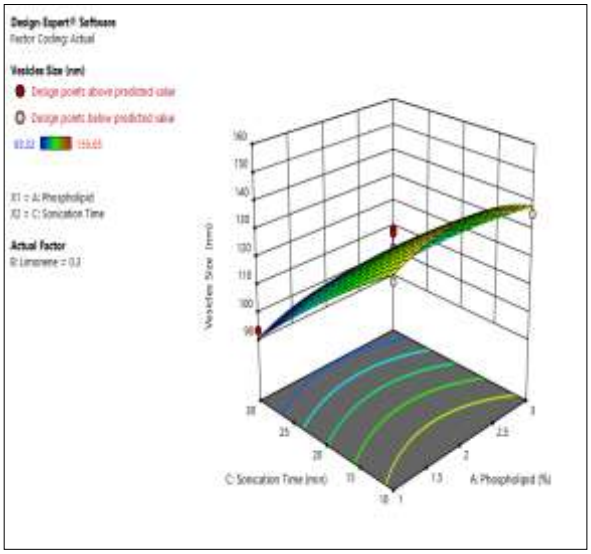


c) Limonene and Sonication time

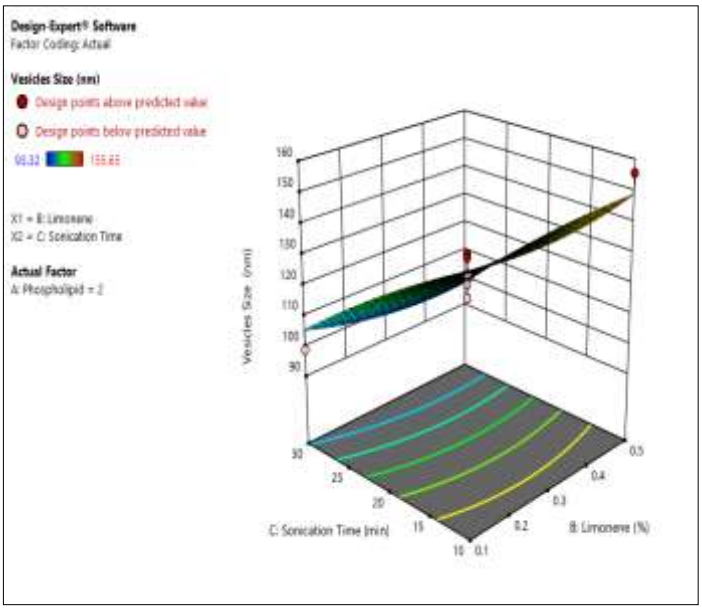
Figure 5: Contour plot for vesicle Size between Limonene and Sonication time



a) Phospholipid and Limonene



b) Phospholipid and sonication time



c) **Limonene and Sonication time**

Figure 6: 3D surface plot for percentage entrapment

Table 4: Experimental results with predicted responses

Formulation	Composition (%) Phosphlipid/ Limonene/ Sonication Time	Response	Actual Value	Predicted value
IF1	3.0/0.3/30	Vesicles Size	95.65	93.37
		Entrapment Efficiency	75.65	76.97
IF2	1.0/0.3/30	Vesicles Size	93.32	90.43
		Entrapment Efficiency	74.65	74.24
IF3	2.0/0.5/30	Vesicles Size	99.45	97.96
		Entrapment Efficiency	76.65	75.87

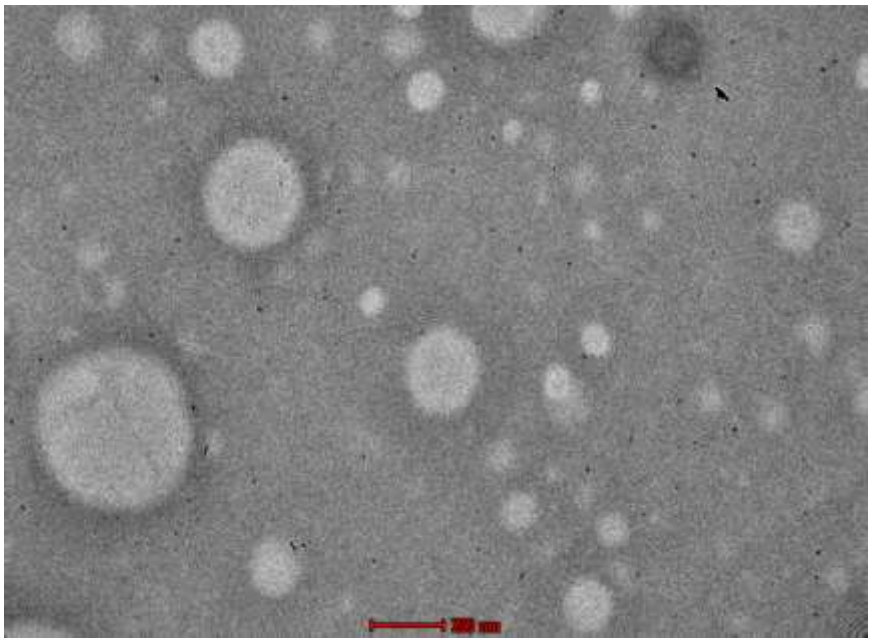


Figure 7.24: Image of Transmission electron microscopy of optimized formulation IGF2

Table 5: Drug release from optimized formulation

S. No.	Time (hr)	Plain Drug	% Cumulative drug release from Invasomes formulation		
			IF1	IF2	IF3
1.	0	0	0	0	0
2.	0.5	19.85	22.23	15.65	11.12
3.	1	39.98	36.65	30.25	20.25
4.	2	59.95	48.89	38.85	30.65
5.	3	78.85	55.65	43.32	40.21
6.	4	95.65	69.98	58.89	55.65
7.	6	-	73.32	63.32	62.23
8.	8	-	89.98	74.45	71.12
9.	10	-	93.32	82.21	76.65
10.	12	-	98.78	86.65	83.32
11.	16	-	99.15	92.23	88.85
12.	20	-	99.25	94.85	90.12
13.	24	-	99.32	96.67	93.32
14.	48	-	99.45	98.85	95.45

CONCLUSION

The results from the study of the invasomes of ketoconazole show that the drug is able to penetrate the bacterial cell membrane and form an invasome. This invasome is able to prevent the growth of the bacteria, thus leading to the death of the bacteria. This highlights the efficacy of ketoconazole in treating a variety of bacterial infections. the formulation of ketokonazole invasomes is a promising new delivery system for this antifungal drug. This method of delivery allows for greater targeted release of the drug, increased stability, and improved bioavailability. Further studies are needed to determine the efficacy and safety of this new delivery system. The formulation of ketokonazole invasomes is a promising new method for treating fungal infections. This method has the potential to provide higher levels of drug delivery, increased bioavailability, and improved efficacy. Further research is needed to evaluate the safety and efficacy of ketokonazole invasomes in humans.

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