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Formulation, optimization and characterization of Betaxolol hydrochloride proniosomes using 3_2 factorial design

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ABSTRACT

The revolution in nanotechnology has lead to the development of various dosage forms such as vesicular drug delivery and in particular liposomes, niosomes, proniosomes, aquasomes, bilosomes etc. The disadvantages exhibited by the liposomes, niosomes can be overcome through introduction of proniosomes which are compact liquid crystalline structures and convert to niosomes upon hydration. The investigation is focused on development and optimization of Betaxolol proniosomes using three square factorial design technique with the aid of design expert 11.0 ® trial version. The optimization technique prefers cholesterol and span 60 as independent variables and drug content, vesicular size, and entrapment efficacy as dependent variables. The design generated total 13 formulations among which F10 exhibited 98.1% drug content and 97.3% of entrapment efficacy. In view of other parameters, F10 exhibits 6.5 pH, 3.8 vesicular size and follows diffusion mechanism with anomalous drug transport. Hence, the obtained results specify that F10 is optimized and can be opted for commercialization.

Keywords: Betaxolol; Three square factorial design; Proniosomes; ocular hypertension; open angle glaucoma.

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INTRODUCTION

Niosomes act as drug reservoirs that enable them to release the drug through its bi layers and provides sustained drug delivery[1-3]. The drug can be targeted to specific areas using minute concentrations through encapsulation thereby declining the rate of drug clearance. The ideology reduced the side effects of the drug molecules and served as a frontier in novel drug delivery system. Niosomes exhibit both hydrophilic and lipophilic properties which enable them to incorporate a variety of drug molecules with varied solubility[4-7]. In addition, Niosomes offer various advantages such as enhanced oral bioavailability, permeability for topical application, and various routes of administration. Further, the vesicles act as a shield in protecting the inside components of niosomes from unfavorable environmental conditions. Hence, this exclusive property of niosomes enables them to encapsulate labile and sensitive drug molecules. Niosomes are found to be osmotically active which enhances the stability of entrapped drug molecule[8-11]. The composition of niosomes includes cholesterol, surfactant and charge inducers among which the non-ionic surfactant are explicitly preferred in formulating the niosomes. The significance of non-ionic surfactants is to decrease the irritation at the site if administration and enhance the entrapment efficacy of drug molecules in proportional to its alkyl chain length. The present investigation incorporates span 60 as surfactant possessing elevated HLB value and leads to formation of bi layer vesicles. The composition also highlights cholesterol, a steroidal metabolite of cell membranes for imparting the rigidity and orientation of bi layers in niosomes. When cholesterol is incorporated with

non-ionic surfactants, it reduces the agglomeration and enhances the stability of niosomes. Cholesterol also prevents the gel to liquid phase transition of bi layers which reduces the drug leakage through vesicles and enhances the entrapment efficacy of drug molecules[12-15]. Apart from the above, the current exploration uses maltodextrin as a carrier that play a significant role in deciding the flexibility and optimization of formulation. The objective of current investigation is to formulate and optimize a stable, biocompatible, biodegradable and non-toxic niosomal formulation and evaluate its various parameters in terms of efficacy and predictability[16-17]. Therefore, the crucial parameters such as drug content, entrapment efficacy, and vesicle size are to be optimized which in turn depend on concentrations of cholesterol and span 60. In order to fulfill the desire criteria, the investigation adopts three square factorial design for optimization of cholesterol and span 60 at three different levels i.e. low, medium, and high using design expert® software trial version and the corresponding formulations are analyzed. In continuation to the above, niosomes possesses enhanced chemical stability and low material cost in comparison to other vesicular drug delivery systems and proved to be useful for commercial production. Hence, the future aspects of niosomes lie in encapsulation of various drug molecules that serves as a promising carrier in achieving desired bioavailability and drug targeting characteristics with decreased toxicity and side effects.

Materials and Methods

Materials used: Betaxolol, cholesterol and span 60 are procured from Yarrow chemicals, Mumbai. Maltodextrin is procured from Finar chemicals, Mumbai. Chloroform and methanol are procured from S.D. fine chemicals, Mumbai.

Formulation of Proniosomes

The proniosomes are prepared by slurry method in which 0.5gm of betaxolol hydrochloride and predefined concentrations of cholesterol and span 60 are dissolved in chloroform and methanol (2:1 ratio). The mixture is incorporated with 0.2gm of maltodextrin and attached to a rotary flash evaporator maintained at 45ºC at 60-70 rpm for complete removal of organic solvent and generates a free flowing product. The product thus obtained is dried for overnight in a desiccator for removal of any traces amount of solvent and named as betaxolol hydrochloride proniosomes. Further, the detailed composition of various formulations carried out in the current investigation is mentioned in table 1 for reference.

Construction of Calibration Curve

The calibration curve for betaxolol hydrochloride is constructed by dissolving 100mg of betaxolol hydrochloride in 100ml of chloroform (Stock solution 1). From this nearly 10ml of solution is withdrawn and diluted with 100ml with chloroform (Stock solution 2). Further, from stock solution 2, the required concentrations are developed as per the beer's range i.e. 5-30µg/ml and absorbance is recorded at 405nm. The details of concentration and its corresponding absorbance are specified in Table 1 and in Figure 1 for reference.

Table 1: Calibration curve of Betaxolol hydrochloride

Concentration (µg/ml)	Absorbance
0	0
5	0.169
10	0.315
15	0.448
20	0.585
25	0.710
30	0.849

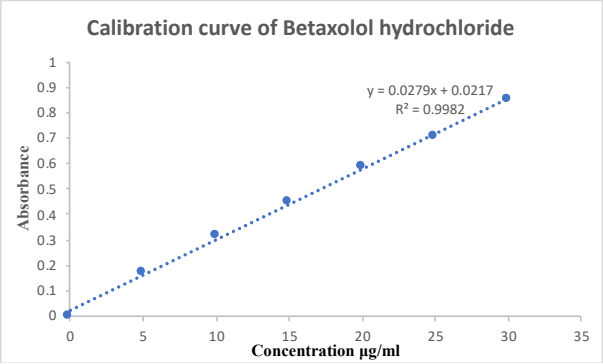


Figure 1: Calibration curve of Betaxolol hydrochloride

Drug Profile and rationality for the preparation of Betaxolol HCl Proniosomes

Betaxolol Hydrochloride is a cardioselective beta-adrenergic receptor blocking agent indicated for the treatment of ocular hypertension and open angle glaucoma. Betaxolol is a BCS class 1 drug possessing high solubility and high permeability and gets easily available at the targeted site producing the required therapeutic effect. However, the enhanced penetrable property of the drug molecule may create elevated drug concentrations at the targeted site and thereby generating a toxic effect. Further, the pharmacokinetic parameters reveal that it exhibits 50% of protein binding and upon oral administration it undergoes first pass metabolism which reduces its bioavailability to 90%. The elimination half life of Betaxolol is 15hours and demand optimized formulation that meets the required specifications in terms of bioavailability and therapeutic effect. Therefore, the present investigation is focused on the development of proniosomal formulation that can release the drug in a sustained manner meeting the predetermined pharmaceutical and biological attributes.

Experimental design and statistical analysis

The factorial design is employed for optimization of betaxolol proniosomes in which the concentrations of

Table 2: Formulation Chart for Betaxolol hydrochloride Proniosomes

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Betaxolol Hcl (mg)	30	30	30	30	30	30	30	30	30	30	30	30
Cholesterol (mg)	50	30	40	30	50	30	40	30	40	50	30	40
Span 60 (mg)	20	30	30	20	30	40	40	40	20	40	20	20
Maltodextrin (mg)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Chloroform (ml)	6	6	6	6	6	6	6	6	6	6	6	6
Methanol (ml)	4	4	4	4	4	4	4	4	4	4	4	4
Phosphate buffer pH 7.4 (ml)	10	10	10	10	10	10	10	10	10	10	10	10

Table 3: Summarization of various independent and dependent variables

Independent Variables	**Levels Used**	**-1**	**0**	**+1**
	A: Cholesterol (mg)	30	40	50
	B: Span 60 (mg)	20	30	40
Dependent Variables	R1: Entrapment Efficacy %EE			
	R3: Drug Content (%)			
Response Variables	Y_1	% drug release in 2 hours		
	Y_2	% drug release in 12 hours		
	Y_3	% drug release in 24 hours		
	Y_4	50% drug release in ($T_{50\%}$)		

Table 4: Indicating the drug content and entrapment efficacy for various formulations

Formulation Code	Cholesterol (mg)	Span60 (ml)	Drug Content (%)	Entrapment Efficacy (%)
F1	50	20	95.6	93.1
F2	30	30	89.3	85.4
F3	40	30	95.1	89.4
F4	30	20	89.1	83.6
F5	50	30	97.3	95.2
F6	30	40	91.5	87.5
F7	40	40	94.2	91.5
F8	30	40	92.1	88.1
F9	40	20	91.5	87.1
F10	50	40	98.1	97.3
F11	30	20	88.4	83.4
F12	40	20	92.6	87.5
F13	40	40	93.8	91.8

Table 5: Comparative In-Vitro drug release studies for various formulations

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
1	12.3	8.1	8.3	6.9	10.4	7.2	9.8	6.7	8.2	14.2	7.9	7.9	10.2
2	23.6	12.5	17.6	7.9	25.8	13.5	19.4	12.8	16.9	29.3	12.1	17.1	21.5
4	34.5	26.6	35.2	23.1	36.7	30.5	36.3	29.5	36.2	41.9	25.8	35.8	44.1
6	48.2	36.5	48.6	30.5	47.5	43.7	50.1	43.2	48.2	57.8	36.2	48.3	58.2
8	58.1	49.7	59.2	42.8	60.2	56.7	61.8	55.7	60.4	66.5	48.8	59.6	70.8
12	83.4	63.7	75.8	60.4	84.8	69.8	76.2	69.1	74.8	87.6	62.5	74.2	81.2
24	91.2	75.8	84.5	72.8	93.5	80.1	87.1	79.8	83.9	95.2	73.1	82.2	89.4

cholesterol and span 60 are considered as the independent variables and entrapment efficacy, drug content are considered as the dependent variables. The effect of these variables on the prepared formulation is assessed at three different levels i.e. low, medium, and high and the possible combinations of variables

in various formulations is depicted in table 3 for reference. Among the generated formulations, and the cumulative drug release at various time intervals such as 2hrs, 12hrs and 24hrs are considered as response variables for which the response surface methodology (RSM) is applied using Design expert® software trial version 11.0 and the corresponding polynomial interactions and quadratic equations are developed with the aid of multiple regression analysis. Therefore, the regression analysis followed the equation $Y= \beta_0+ \beta_1A+ \beta_2B+ \beta_3AB+ \beta_4A_2+ \beta_5B_2+ \beta_6A_2B + \beta_7AB_2 + \beta_8A_2B_2$ in which β_0 signifies the intercept, A and B are the coded variables with respect to independent variables, and A_2B_2 indicates the interaction between the quadratic terms. In a similar fashion, the 2-dimentional counter plots were generated using Design expert® software trial version 11.0 which are quite useful in understating the interactions between independent variables and the possible outcomes.

Table 6: Comparative in-vitro -drug release studies for various formulations at 2 hrs, 12 hrs, and 24 hrs

Formulation	Factorial Amount (mg)		Rel_2 h (%)	Rel_{12} h (%)	Rel_{24} h (%)
	Cholesterol	Span 60			
F1	50	20	23.6	83.4	91.2
F2	30	30	12.5	63.7	75.8
F3	40	30	17.6	75.8	84.5
F4	30	20	7.9	60.4	72.8
F5	50	30	25.8	84.8	93.5
F6	30	40	13.5	69.8	80.1
F7	40	40	19.4	76.2	87.1
F8	30	40	12.8	69.1	79.8
F9	40	20	16.9	74.8	83.9
F10	50	40	29.3	87.6	95.2
F11	30	20	12.1	62.5	73.1
F12	40	20	17.1	74.2	82.2
F13	40	40	21.5	81.2	89.4

Table 7: Comparison of various kinetic parameters for prepared formulations

Formulation Code	Kinetic Parameters				
	Zero Order	First Order	Higuchi	Korsemeyer peppas	
	Regression Coefficient	Regression Coefficient	Regression Coefficient	Regression Coefficient	"n" Values
F1	0.828	0.445	0.955	0.968	0.654
F2	0.856	0.512	0.956	0.966	0.766
F3	0.800	0.466	0.944	0.942	0.762
F4	0.884	0.564	0.947	0.949	0.846
F5	0.828	0.447	0.955	0.947	0.689
F6	0.814	0.496	0.942	0.946	0.812
F7	0.825	0.452	0.951	0.949	0.718
F8	0.820	0.505	0.942	0.946	0.835
F9	0.794	0.465	0.941	0.937	0.767
F10	0.785	0.402	0.952	0.949	0.609
F11	0.846	0.511	0.952	0.963	0.766
F12	0.785	0.462	0.938	0.933	0.768
F13	0.738	0.421	0.922	0.916	0.710

Evaluation and characterization of Betaxolol hydrochloride Proniosomes

Morphological and Vesicular size analysis

The vesicular size is determined by using optical microscopy method in which a digital camera is fitted which is capable of capturing the photograph of the prepared formulation under 100X magnification. The procedure involves spreading a thin layer of the film on the microscope slide, covered with a cover slip and the resultant is placed under the microscope. In addition, the formulation image is adjusted as per the requirement and the dimensions are recorded accordingly.

Drug Content: The drug content is assessed by subjecting the specific quantity of the sample in a volumetric flask containing 50ml of phosphate buffer pH 7.4. The volumetric flask is subjected for magnetic stirring for 24hours and the corresponding samples are withdrawn which are diluted as per the beers range and the drug content is determined. The following formulated is accessed for determining the drug content of the prepared formulations:

$$\% \text{ Drug Content} = \frac{\text{Absorbance}}{\text{Slope}} \text{ X (Dilution Factor) X } \frac{1}{1000}$$

Entrapment Efficacy: The entrapment efficacy is assessed through centrifugation method in which the predetermined quantity of the formulation is placed in the ultra centrifuge and subjected for centrifugation at 10,000rpm for 15minutes. From the resultant the supernatant liquid is separated, diluted according to Beer's range and the corresponding drug entrapment is determined at 405nm using UV spectrophotometric method. Further, the entrapment efficacy is determined using the following equation:

$$\% \text{ Entrapment Efficacy} = \frac{\text{Amount of drug entrapped}}{\text{Amount of drug added}} \text{ X } 100$$

Determination of pH: The pH of the prepared formulation is determined by using digital pH meter which was initially calibrated using standard phosphate buffer pH 7.4. The electrode is inserted into the formulation whose pH is to be determined and the reading is recorded at room temperature. The procedure is repeated for three times and the average pH value is recorded.

In-vitro drug release studies: The in-vitro drug release studies are performed by using USP type 2 dissolution apparatus in which the dissolution medium containing 900ml of phosphate buffer pH 7.4 is maintained at 37±0.5ºC with paddle speed of 50rpm throughout the process. In between predetermined aliquots of sample is withdrawn and the same is replaced with fresh fluid and the cumulative drug release is determined at 405nm spectrophotometrically

Release Kinetics: The mechanism of the drug release from the prepared formulation is found out through interpretation of in-vitro release data to different kinetic models such as Zero order, First order, Higuchi, and Korsemeyer-peppas. The basic criteria for adaptation of specific value depend on its goodness of fit and regression coefficient value.

RESULTS AND DISCUSSION

Drug content and entrapment efficacy

In the current investigation various formulations were generated by altering the ratio of cholesterol and span 60 and analyzed their effect on entrapment efficacy and drug content. The results reveal F10 contains 98.1% drug content and 97.3% entrapment efficacy and considered as optimized. In general, as per the theoretical background the drug content and the entrapment efficacy enhances proportionally with cholesterol concentrations up to a certain extent and then declines. This might be due to the fact that when incorporated along with surfactants it provides rigidity and orientation order through alignment of OH groups towards aqueous phase and aliphatic chain towards hydrocarbon chain of the surfactant. Therefore the rigidity occurs through the simultaneous arrangement of steroidal skeleton with surfactant molecules thereby restricting the free movement of the hydrocarbons. The above mentioned theory is favored up to a certain concentration levels and the same is generated in formulation F10 and any further increase in the concentrations of cholesterol beyond 50mg has lead to the rapid fall down of the above mentioned parameters. Since, the optimized formulation is based on enhanced entrapment efficacy and drug content, the two are quite superior in F10 when compared to the rest of the formulations. Hence, in view of the above discussion it can be inferred that F10 is quite optimized and meets the required criteria. The results in related to drug content and entrapment efficacy are depicted in table 4 for reference.

In-vitro drug release study: The drug release studies for the prepared formulations are assessed for the prepared formulations as per the procedure described above and the results are predicted in table 5 for reference. The results reveal a linear release and a maximum drug release of 95.2% for F10. Although there are other formulations such as F5 and F1 possessing 93.5% and 91.2% drug release, they are not considered as optimized because they possess decreased entrapment efficacy and drug content when compared to F10. Further, the theoretical background for F5 and F1 to exhibit a decreased drug release might be due to altered cholesterol: Span ratio. It is believed that as the concentration of surfactant is increased, the drug release characteristics will increase proportionally and the same is observed for generated formulations. Further, the presence of penetration enhancer in the formulations show a significant effect on the drug release characteristics and furthering, the increase in cholesterol concentration makes the vesicles much rigid and thereby preventing the drug leakage. In both F5 and F1 the elevated concentrations of cholesterol generates enough rigidity of vesicles, while the considerable concentrations of span make the vesicles fluffy and enhance the desired characteristics. Hence, the optimization of both concentrations generated F10 that meet the desired criteria. In connection to the above, a comparative drug release studies for prepared formulations at various time intervals such as 2hrs, 12hrs, and 24hrs is studied and the same is predicted in table 6 for reference.

Release Kinetics and Statistical analysis: The obtained in-vitro drug release data is subjected for various kinetic models such as zero order, first order, Higuchi, and Korsemeyer peppas model for determining the type of drug release and its kinetic profile from the Proniosomes (table 7) (Figure 2-13). The results followed a linear relationship and generated higher R_2 values for zero order in comparison to first order kinetics which confirms that the formulation follows first order kinetics. Further, the Higuchi values range from 0.938 to 0.956 which confirms that the drug follows diffusion mechanism. The Korsemeyer peppas data predicts the "n" values from (>0.5 and <1) which specifies non-fickian diffusion (anomalous drug transport). The generated data is subjected to ANOVA studies using design expert 11.0® trial version software and the polynomial equations in terms of coded equations are generate in which the positive sign indicates that there is an increase in the dependent variables on simultaneous in the independent variables and vice versa.

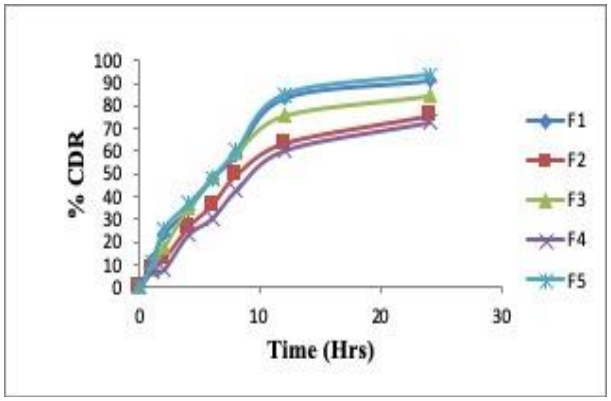


Figure 2: Zero Order release from F1 to F5

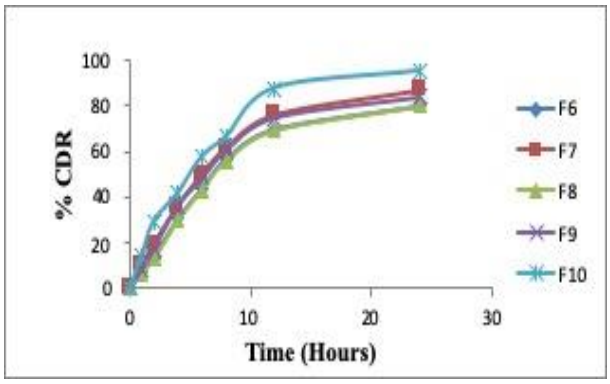


Figure 3: Zero Order release from F6 to F10

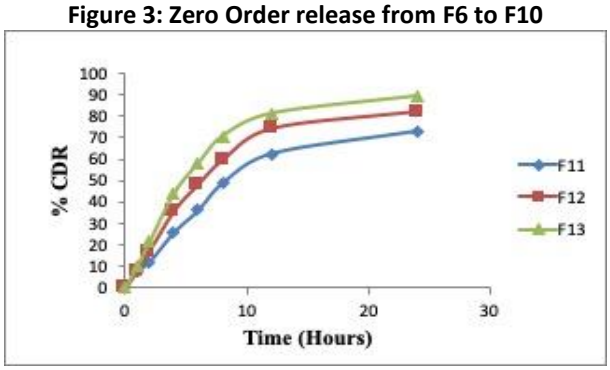


Figure 4: Zero Order release from F11 to F13

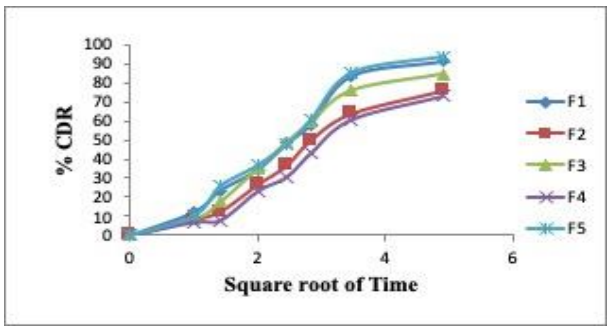


Figure 5: Higuchi model from F1 to F5

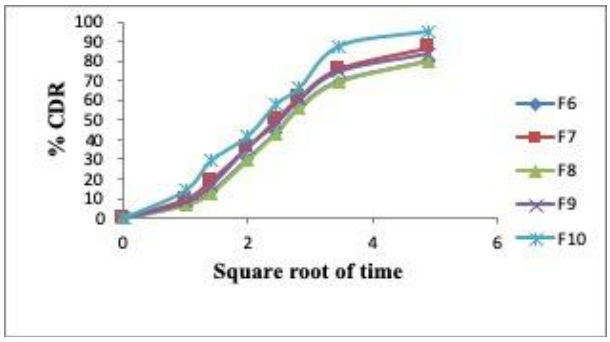


Figure 6: Higuchi model from F6 to F10

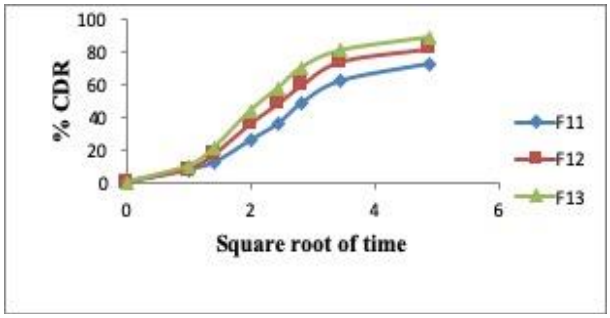


Figure 7: Higuchi model from F11 to F13

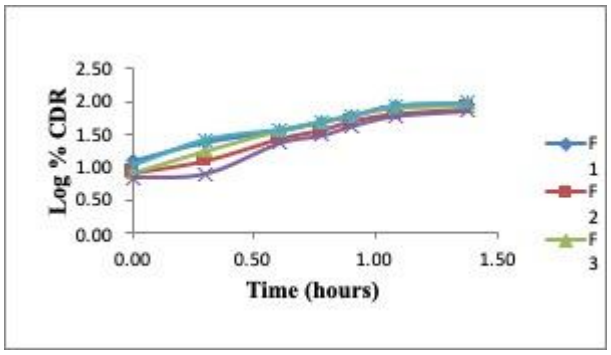


Figure 8: First Order release from F1to F5

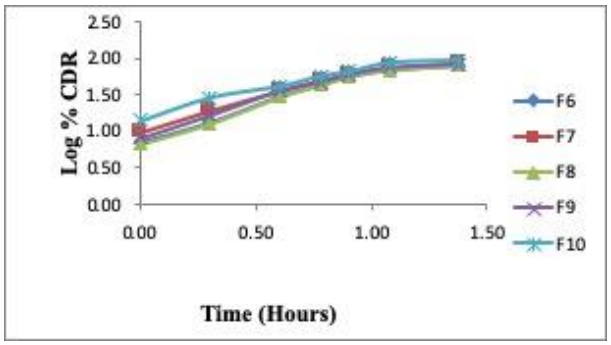


Figure 9: First Order release from F6 to F10

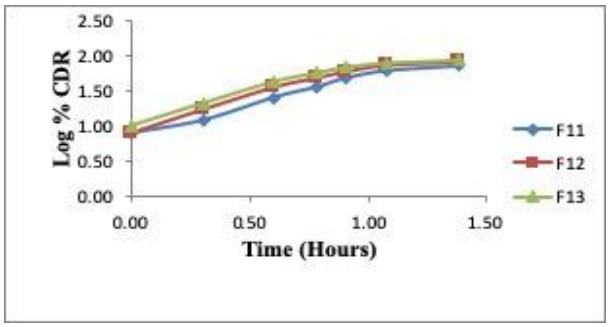


Figure 10: First Order release from F11 to F13

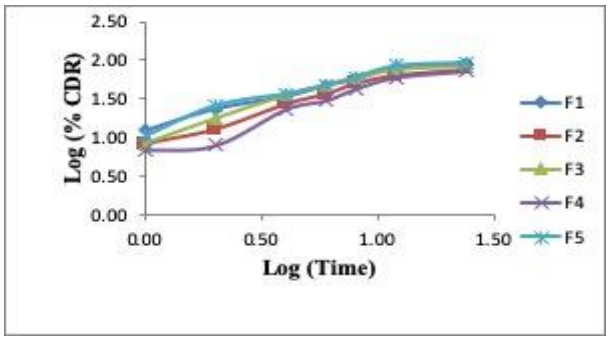


Figure 11: Korsemeyer Peppas model from F1to F5

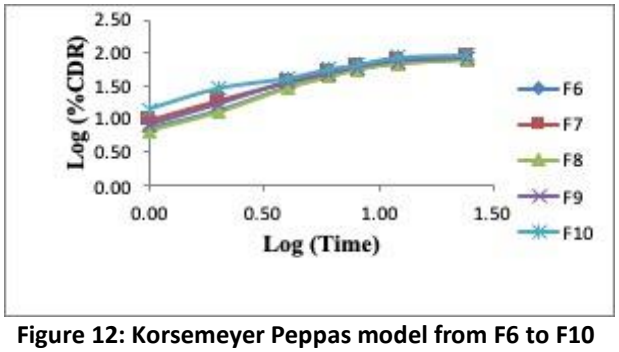


Figure 12: Korsemeyer Peppas model from F6 to F10

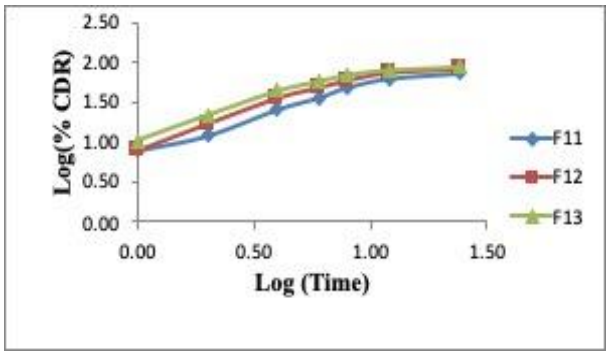


Figure 13: Korsemeyer Peppas model from F11 to F13

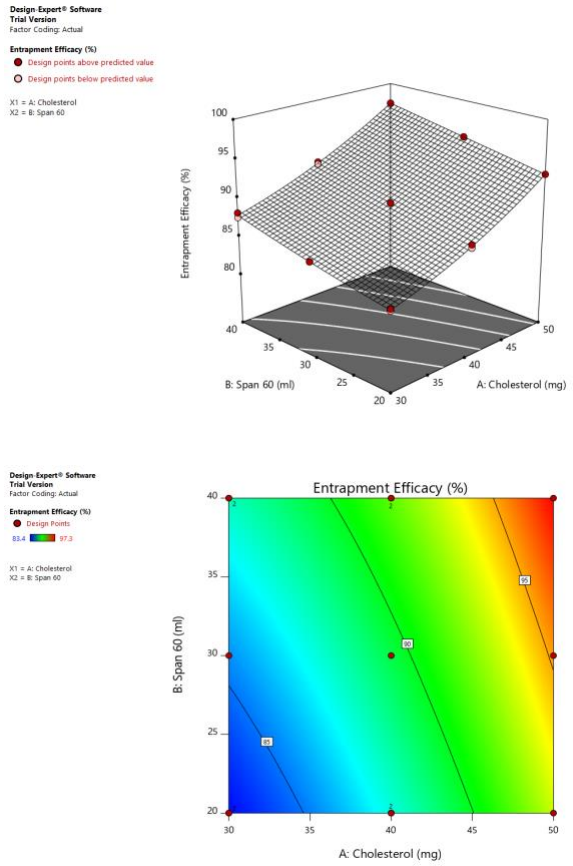
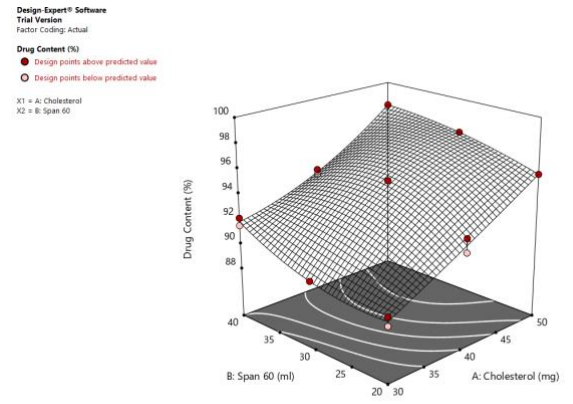


Figure 14: 2-D Counter Plot for Drug Entrapment Efficacy



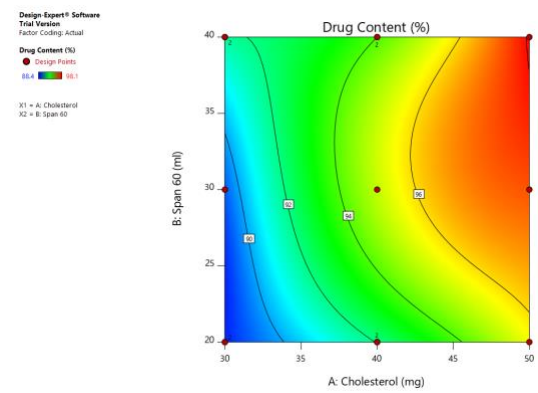


Figure 15: 2-D Counter Plot for Percentage Drug Content

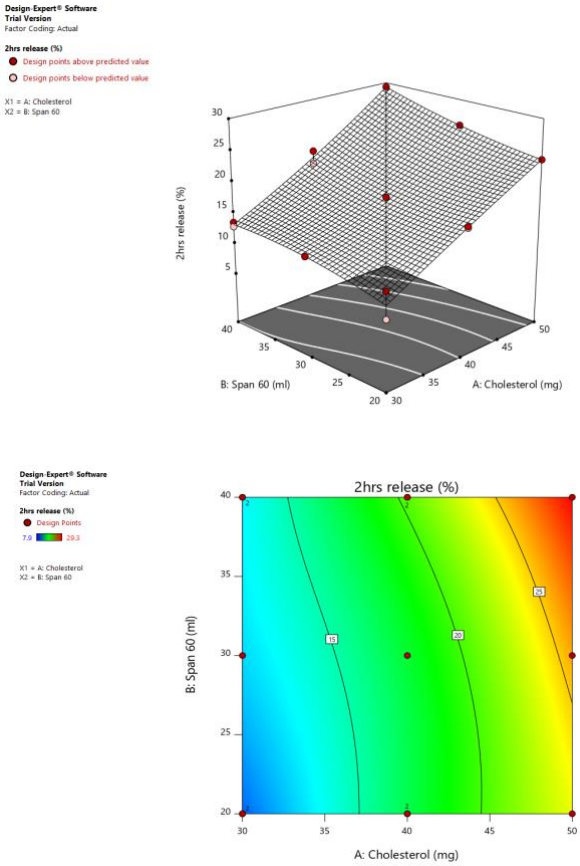
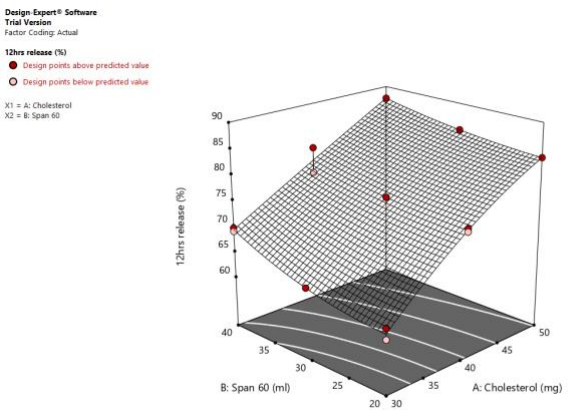


Figure 16: 2-D Counter Plot for 2hrs Drug Release



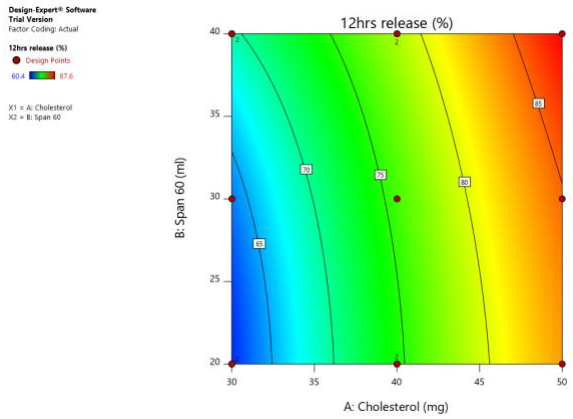


Figure 17: 2-D Counter Plot for 12hrs Drug Release

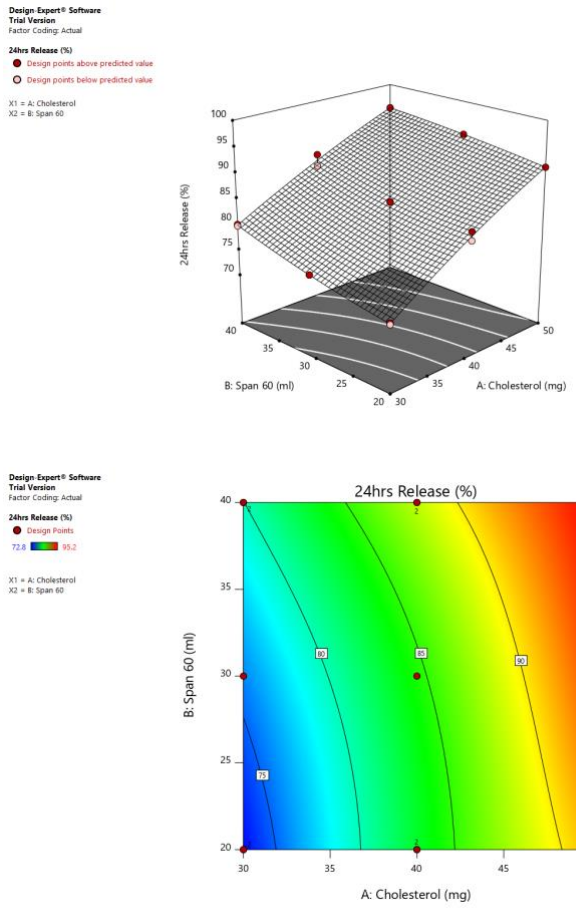


Figure 18: 2-D Counter Plot for 24hrs Drug Release

Polynomial Equation in terms of coded variables

For Entrapment Efficacy: Y = 89.40 +4.90A +2.18B -0.0250AB +0.9000A_2 +0.0750B_2 -0.0500A_2B -0.1250 AB_2 +0.0500A_2B_2

For Drug Content: Y = 95.10 +4.00A +0.9750B -0.1375AB -1.80A_2 -2.07B_2 +0.4125A_2B -0.7125AB_2 +2.34A_2B_2

For 2hrs release Y = 17.60 + 6.65A + 1.72B + 0.6375AB + 1.55A_2 + 1.13B_2 + 0.4875A_2B + 0.7875AB_2 – 1.26A_2B_2

For 12hrs Release Y = 75.80 + 10.55A +2.10B -0.95AB – 1.55A_2 +0.80B_2 +0.95A_2B - 5250AB_2 +0.4250A_2B_2

For 24hrs release Y = 84.50 +8.85A + 2.50B -0.75AB +0.15A_2 +1.15B_2 +0.15A_2B -0.4750AB_2 -0.9750A_2B_2

pH and Vesicle analysis: The pH and the corresponding vesicle size of various prepared formulations are determined using standard pH meter and vesicle analysis through optical microscopy technique and the results are predicted in table 8 for reference. The results specify the pH range from 6.1 to 6.7, and vesicle range from 3.1 to 3.8 which make sense that the prepared formulations possess optimized pH and small unilamellar vesicles. At the same time the morphological characteristics of the particles specify that they are nearly spherical in shape with discrete boundaries.

Table 8: Comparison of pH and vesicle size for various formulations

Formulation Code	pH	Vesicle size
F1	6.4	3.4
F2	6.5	3.6
F3	6.2	3.8
F4	6.3	3.5
F5	6.5	3.1
F6	6.7	3.6
F7	6.1	3.5
F8	6.5	3.8
F9	6.3	3.4
F10	6.5	3.8
F11	6.2	3.5
F12	6.1	3.7
F13	6.5	3.6

CONCLUSION

Niosomes serve as a promising drug delivery for various drug molecules because of their explicit properties such as enhanced chemical stability, purity, low material cost, capability to incorporate variety of molecules. The extreme advantages have inspired to formulate and optimize the niosomal formulation of betaxolol which is drug of choice in open angle glaucoma. The research adopts three square factorial design for the optimization of independent variables such as cholesterol and span 60 based on which the dependent variables such as drug content, entrapment efficacy, and vesicle size are estimated. The design developed total 13 formulations among which F10 exhibits 98.1% drug content and 97.3% of entrapment efficacy which is found to be quite optimized and meets the required criteria. The other parameters such as release kinetics reveal that F10 follows first order kinetics with diffusion mechanism. The ANOVA studies and polynomial equations in terms of coded variables for dependent variables signify that there exist a direct proportional relationship between the dependent and independent variables. Thus in view of above

discussion, it can be conclude that F10 is optimized formulation that meets the required criteria.

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