INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.7 | Issue 3 | July- Sep -2018

Journal Home page: www.ijpar.com

Research article *Open Access*

Impact of three square factorial design in formulation and characterization of mianserintransferosomes

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ABSTRACT

Objective

The current investigation deals with formulate and characterize the Mianserintransferosomes using 3^2 factorial design.

Method

Mianserin is a BCS class II drug and is widely preferred for the antidepressant action. The transferosomes are developed using thin film hydration method by varying the concentrations of lecithin: span 80 and Mianserin. A total of 9 formulations were developed by considering the ration of lecithin: span 80 and Mianserin as independent variables and assigned as X1 and X2 respectively and entrapment efficacy, drug content, and vesicle size as the dependent variables. Further, the results were subjected to response surface methodology using sigma plot® software and the statistical equations were drawn out.

Results

The generated results clarifies that the current formulations meet the pharmacopoeial standards and justifies F5 as the optimized formulation. When subjected to kinetic modeling F5 exhibited first order release kinetics with non-Fickian diffusion. Further the development of polynomial equations for dependent variables through step-wise backward non-linear regression analysis revealed the interaction between the selected variables and their effect on interchanging them.

Conclusion

The F5 is considered as optimized formulationand follows first order, and Higuchi kinetics, and the mechanism of drug release is found to be non-Fickian diffusion anomalous transport.

Keywords: 3^2 factorial design, Transferosomes, Mianserin, Thin film hydration, Span 80, Cholesterol.

INTRODUCTION

Mianserin belongs to piperazino-azepine group of compound which is not chemically related to tricyclic antidepressants. The structure is devoid of basic side chain and is mainly responsible for anticholinergic activity of tricyclic antidepressants. The exact mechanism of action is not well understood and believed that it blocks the alpha adrenergic, histamine H_1 and some type of serotonin receptors [1]. Mianserin in available in the form of tablet dosage form and gets rapidly absorbed through oral administration. The peak plasma levels are attained within 3 hrs of administrations and nearly 95% of the drug exhibits plasma protein binding. The bioavailability of the drug is approximately 20% and exhibits an elimination half life of 21-61hrs[2,3]. The steady state plasma levels are attained within 6 days and is extensively metabolized and eliminated through urine and feces within 7 to 9 days [4]. Therefore, there occurs a necessity to enhance the bioavailability of the drug and reduce the afore mentioned limitations. The current investigation is focused on the development of Mianserin transferosomes that finds to be safe, convenient and offers several advantages over conventional ones such as reduced gastrointestinal incompatibility, variable gastrointestinal absorption, avoidance of first pass metabolism, enhanced bioavailability, reduced frequency of administration, enhanced patient compliance. There are numerous drug delivery systems that can meet the above criteria such as nanosphers, microcapsules, micro particles, Solid lipid nanoparticles, vesicular drug delivery systems etc… The current exploration highlights transferosomes which belongs to vesicular drug delivery system and offers a versatile permeation and delivery of various drug molecules[5]. Transferosomes are the artificial vesicles that are several orders of magnitude and possess enhanced deformability when compared to the liposomes. In case of liposomes the deformability can be achieved through selection of appropriate surfactants and the same can be overcome using transferosomes which can squeeze themselves through the intercellular sealing lipid of the stratum corneum. Hence, the resultant imparts enormous flexibility and minimizes the vesicular rupture when allowed to pass through the biological membranes [6, 7]. Currently, the investigators are facing challenge to trace out an optimum combination of variables that can generate an optimized formulation. The present investigation is focused on designing a set of experimental conditions that measures the response variables, mathematically fits the data, performs a suitable statistical test for assuring the best possible model selected, and investigates the optimized value of various independent variables that generates the best possible response. In justification to the above theory, we made an attempt to develop Mianserin transferosomes which is a BCS class II drug and optimize those using 3^2 factorial design with the aid of Sigma plot® V12 software. Further, we studied the evaluation parameters such as entrapment efficacy, drug content, vesicular size and *in-vitro* drug release studies and interpreted the same using 3D mesh and counter plots through Sigma plot® V12 software.

MATERIALS AND METHODS

Materials

Mianserin was obtained fromYarrow pharmaceuticals, Ahmedabad, India.Lecithin and Span 80 were obtained from Yarrow chemicals, Ahmedabad, India. Chloroform and Ethanol (A.R) were obtained from Finar chemicals, Mumbai, India.

Preparation of mianserin loaded transferosomes

The Mianserin loaded transferosomes were prepared by centrifugation method in which the appropriate quantities of lecithin, Span and Mianserin are dissolved in a suitable quantity of chloroform and ethanol mixture as mentioned in table 2. The organic solvents are removed by rotary evaporation under reduced pressure at 40ºC and the traces of solvent remaining are removed under vacuum. The deposited film is hydrated with phosphate buffer solution (pH 7.4) by centrifugating at 60 rpm for 1hour at room temperature. At room temperature the vesicles are swollen for nearly 2 hours and the resultant is further sonicated to obtain the vesicles of suitable size[8].

Table 1: Formulation chart

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Mianserin (mg)	20	15	10	20	15	10	20	15	10
Lecithin (mg)	95	95	95	85	85	85	75	75	75
Span 80 (mg)	5	5	5	15	15	15	25	25	25
Chloroform (ml)	3	3	3	3	3	3	3	3	3
Ethanol (ml)	2	2	2	2	2	2	2	2	2

Drug profile and rationality for transferosomes

The present investigation is focused on the Mianserin transferosomes that can generate a sustain release effect and can ultimately enhance the pharmacokinetic parameters of the drug. Mianserin is atypical antidepressant which is used for the treatment of depression. The insight on various pharmacokinetic parameters of Mianserin reveals that the drug gets readily absorbed from the gastrointestinal tract and undergoes first pass metabolism reducing its bioavailability by 70%. Further, it is extensively bounded to plasma proteins and exhibits 20 – 30% bioavailability. It reveals elimination half life of 21- 61 hrs and excreted 4 – 7% in urine and 14 – 28% in feces[2,9].

Therefore, a suitable formulation that can enhance the pharmacokinetic parameters is to be designed and the current investigation is focused on the above criteria through a specific design that meets the above requirements.

In order to fulfill the current strategy, various response surface methodologies (RSM) utilizing a polynomial equation are preferred. Currently, various RSM's such as factorial design, central composite design, Box-Behnken design, and D-optimal design are available for successfully carrying out the investigation. Apart from these, the RSM is employed only when a few significant factors are involved in optimization and demands less time and experimentation trials. Hence, The RSM's can be more economical and effective than the traditional methods of formulation design.

The investigation on Mianserin transferosomes outlines the optimization of Mianserin and lecithin concentration that exhibits a profound effect on the release characteristics of the drug. Among the above mentioned RSM's a 3^2 factorial design is employed for the investigation of the effect of two independent variables i.e. Mianserin and lecithin: span 80 on the dependent variables such as such as entrapment efficacy, vesicle size, drug content, and the drug release at 2, 12 and 24 hrs.

Experimental design and statistical analysis

A factorial design was employed as per the standard protocol for the generation of optimized formulation of transferosomes. The concentration of lecithin: Span 80 and Mianserin were considered as the independent variables and entrapment efficacy, vesicle size, and drug content are considered as the dependent variables. The effect of these variables is studied at three levels i.e. high, medium, low and the remaining process variables are kept constant throughout the study. The summarization of factor combinations is depicted in table 2 and Rel_2 h (%) relates to the amount of drug released in 2hrs and Rel_{12} h (%)(%) relates to the amount of drug released in 12hrs and (%) and Rel_{24} h (%) relates to the amount of drug released in 24hrs and are considered as the response variables. The response surface method (RSM) computations for the current optimized study is generated by using Sigma Plot10® software and various polynomial interactions and quadratic terms were generated using multiple linear regression analysis. The generalized multiple linear regression analysis is represented by using the following equation:

$$Y= \beta_0+ \beta_1A_1+ \beta_2B_2+ \beta3A_1B_2+ \beta_4A^2+ \beta_5A^2+ \beta_6AB^2+ \beta_7A^2B$$

Where, β0 is the intercept representing the arithmetic average of all quantitative outcomes of 13 runs; $\beta_1 - \beta_7$ are the coefficients computed from the observed experimental response values of Y; A and B are the coded levels of the independent variable(s). The term A_2 B_2 and A_2 represent the interaction and quadratic terms, respectively, statistical validity of the polynomials was established on the basis of ANOVA provision in the Sigma Plot10® software. Two dimensional (2-D) Contour plots, Figures 3 and 4 were constructed based on the model polynomial functions using Sigma Plot10® software. These plots are very

useful to see interaction effects of the factors on the responses.

Table 2: Factor combinations of independent variables as per the experimental design

	Levels Used		
Independent Variables	**-1**	**0**	**+1**
A: Lecithin: Span 80	75:25	85:15	95:5
B: Mianserin	10	15	20
Dependent Variables			
R1: Entrapment Efficacy %EE			
R2: Vesicle size (µm)			
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 ($T_{50\%}$)		

CHARACTERIZATION OF TRANSFEROSOMES

Morphological and size of vesicles

An optical microscope is fitted with a digital camera that can capture the photograph of the prepared formulation under the magnification of 40X. A thin layer of the prepared formulation is spread on a glass slide and a cover slip is placed on the glass slide and observes under the microscope. The image of the formulation is adjusted according to the requirement and the necessary dimensions are measured accordingly [10].

Drug content

The drug content was determined by taking one ml if the transferosomes and diluting the resultant to 100ml with 0.1M HCl. Aliquots of 5ml are withdrawn and diluted to 25ml with distilled water and the concentration was determined at 278 nm spectrophotometrically [11].

Entrapment efficacy

The entrapment efficacies of the transferosomes are prepared by using centrifugation method in which the transferosomes are placed in the ultracentrifuge and operated at 10,000rpm for 10min. The supernatant layers are separated and the drug content is determined after suitable dilution at 278 nm using UV spectrophotometer. The entrapment efficacies are calculated by using the formula:

$$\% \text{ Entrapment Efficacy} = \frac{X1-X2}{X1} \text{ X } 100$$

Where, X1 is considered as amount of Mianserin added initially and X2 is considered as amount of Mianserin determined in the filtrate through spectrophotometrically and (X_1-X_2) represents the amount of Mianserin entrapped in the formulation[12].

RESULTS AND DISCUSSION

Evaluation of vesicles

Morphological and size of vesicles

The morphological characteristics reveal that the vesicular size varies from 3.08 to 3.89 as shown in table 3 which signifies small unilamellar vesicles.

Drug content and entrapment efficacy

Among the 9 formulations, F1 exhibited the highest drug content which might be due to increased lecithin concentration, drug concentration and decreased surfactant concentration. As the concentration of the lecithin increases, the capacity of the drug to diffuse through the core decreases and the drug content increases. While in the other formulations such as F2 and F3, the concentration of lipid and surfactant remained the same, but the drug concentration is reduced which might be the reason for decreased drug content. The above mentioned theory can be extended to the remaining formulations which forms a basis for deviations in the generated results. In consideration to the entrapment efficacy, it depends on the edge activator concentration which is span 80. As the

concentration of edge activator increases, the entrapment efficacy increases to a certain extent and further it will leads to a decline in the entrapment efficacy. The major reason for this is related to the flexibility of the vesicles by the increased concentration of edge activator, causing drug leakage. Therefore, correlating the exiting theory the rest of formulations, the current justification for optimized formulation is based on the entrapment efficacy and F5 signifies a highest entrapment efficacy and considered as optimized.

Table 3: Response parameters for the transferosomes formulation prepared as per the three square factorial design

	Independent Variable		Drug Content	Dependent Variable	
Formulation Code	**A**	**B**		**%EE**	**Vesicle Size**
F1	0.00	1.00	92.5	65.59	3.47
F2	-1.00	1.00	90.0	68.47	3.08
F3	-1.00	0.00	89.0	67.54	3.25
F4	1.00	1.00	87.0	79.36	3.84
F5	-1.00	-1.00	86.0	83.84	4.54
F6	0.00	-1.00	85.0	73.62	3.48
F7	1.00	-1.00	84.0	59.26	3.89
F8	1.00	0.00	83.5	61.25	3.73
F9	0.00	0.00	84.2	65.48	3.24

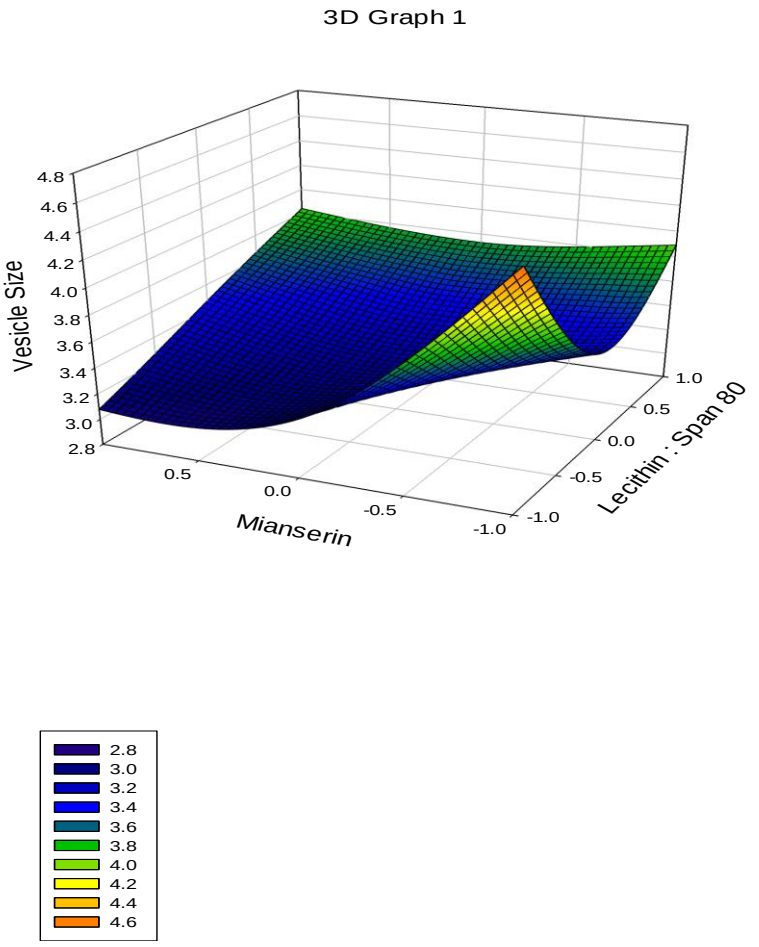

Figure 1: 3D graph showing response parameters for the effect of Lecithin: Span80 on the Vesicle size of transferosomes

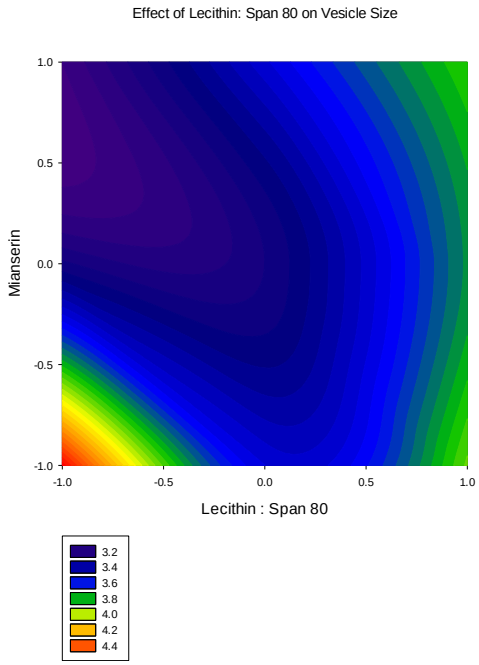

Figure 2: Effect of Lecithin: Span80 on the vesicle size of transferosomes

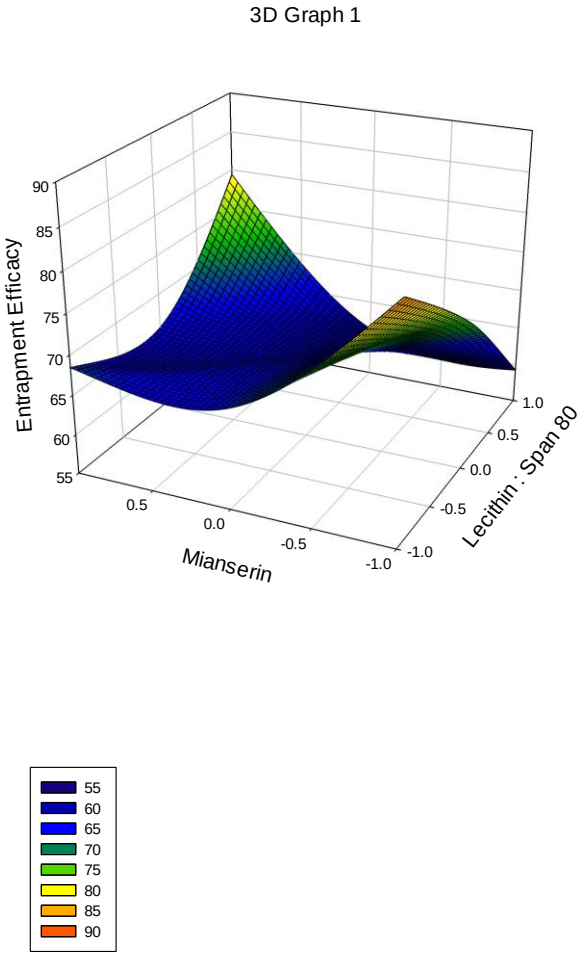

Figure 3: 3D graph showing response parameters for the effect of Lecithin: Span80 on the Entrapment efficiency of transferosomes

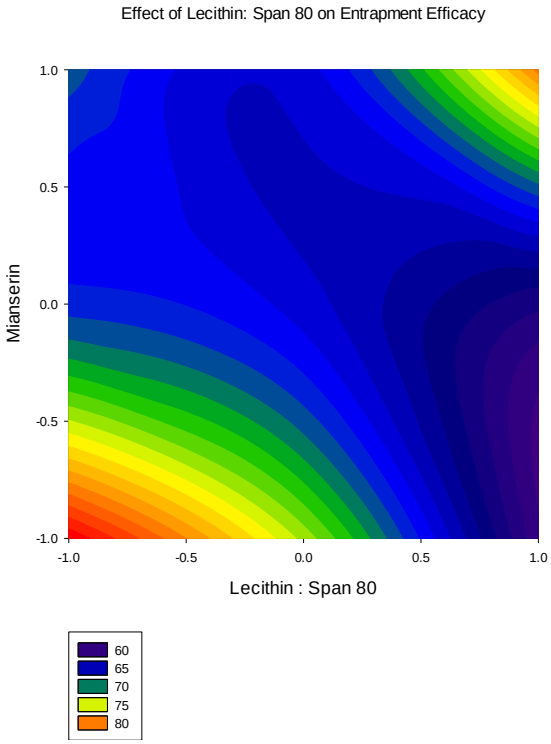

Figure 4: Effect of Lecithin: Span80 on the Entrapment efficiency of transferosomes

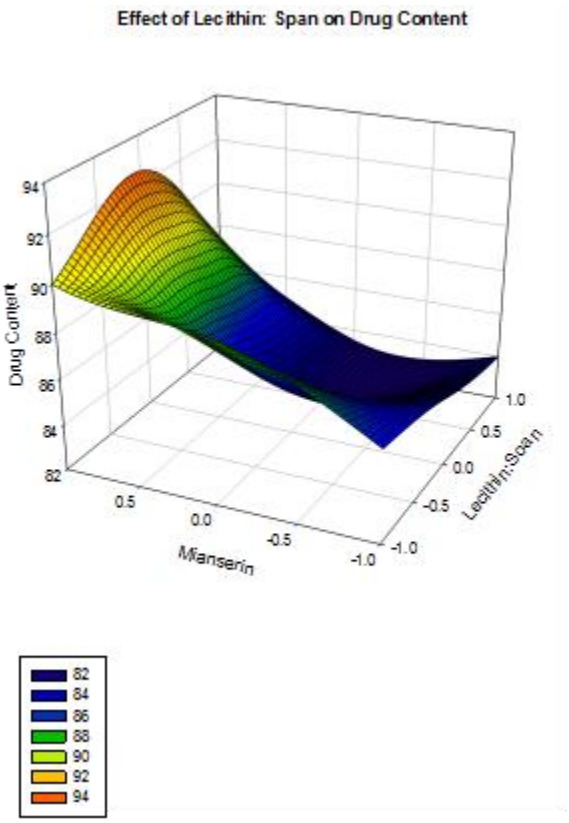

Figure 5: 3D graph showing response parameters for the effect of Lecithin: Span80 on the Drug content of transferosomes

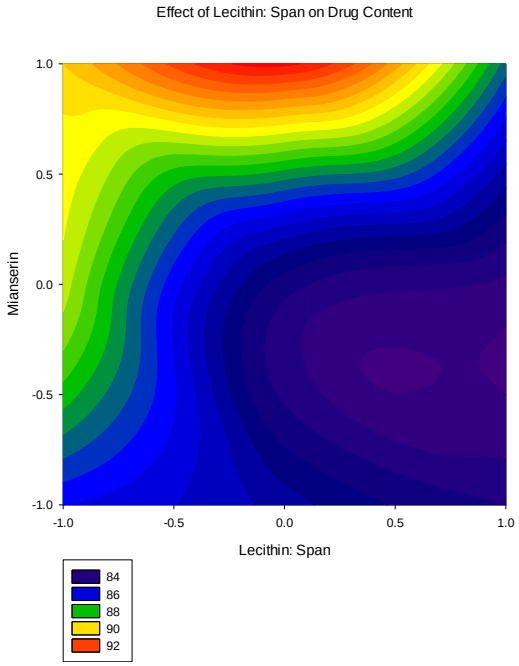

Figure 6: Effect of Lecithin: Span80 on the Drug content of transferosomes

In-vitro drug release studies

The in-vitro drug release studies are depicted in table 4 which clarifies that the drug release is inversely proportional to the concentration of lipid. The results clarify that F5 formulation has a maximum drug release of 98.3% in 24 hrs and F3 formulation has a minimum drug release of 83.4% in 24 hrs. Therefore in terms of sustained release activity F9 proves to be the optimized formulation. But when the other parameters such as entrapment efficacy, drug content are compared, it shows poor results because of its decreased lipid concentration which is the main reason for declined results. Even though the lecithin and span concentration is increased in F1 to F3 formulations it showed a decreased entrapment efficacy because of the drug leakage. Hence, in view of the above discussion it can be conferred that F5 is considered as an optimized formulation. In connection to the above, the drug release to various models justify that

Table 4: In-Vitro cumulative drug release:

Time (hrs)	% Cumulative Drug Release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	15.7	16.4	16.9	17.2	17.8	16.2	15.5	14.8	13.7
2	25.4	24.7	23.2	27.8	26.9	21.5	24.5	22.1	21.4
4	36.7	35.4	33.8	39.5	38.2	31.8	35.6	33.2	32.8
6	45.1	44.2	43.5	49.6	51.2	42.3	47.5	45.8	42.7
8	65.4	64.5	63.8	69.7	71.5	56.6	63.7	62.4	61.8
12	85.8	84.7	83.4	85.4	86.2	63.4	84.3	82.5	81.2
24	96.7	95.4	96.5	98.2	98.3	85.1	95.5	93.6	83.4

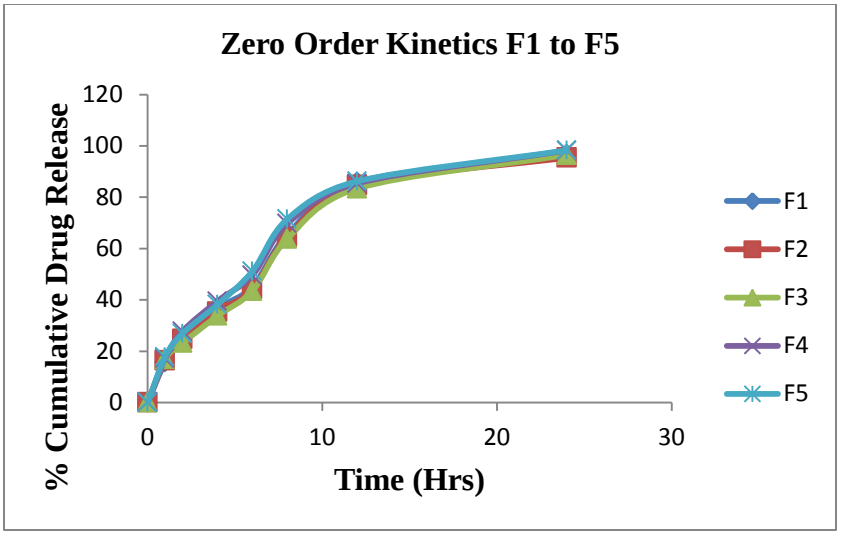

Figure 7: Zero order kinetics (F1-F5)

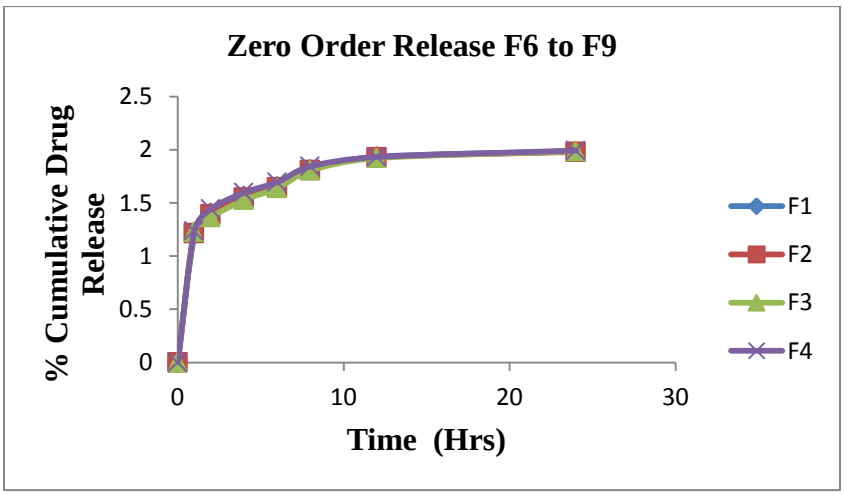

Figure 8: Zero order kinetics (F6-F9)

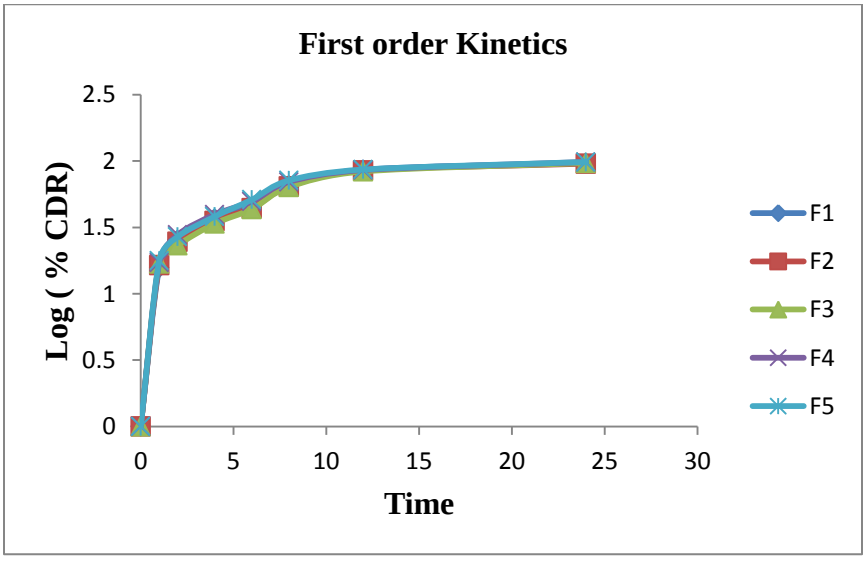

Figure 9: First order kinetics (F1-F5)

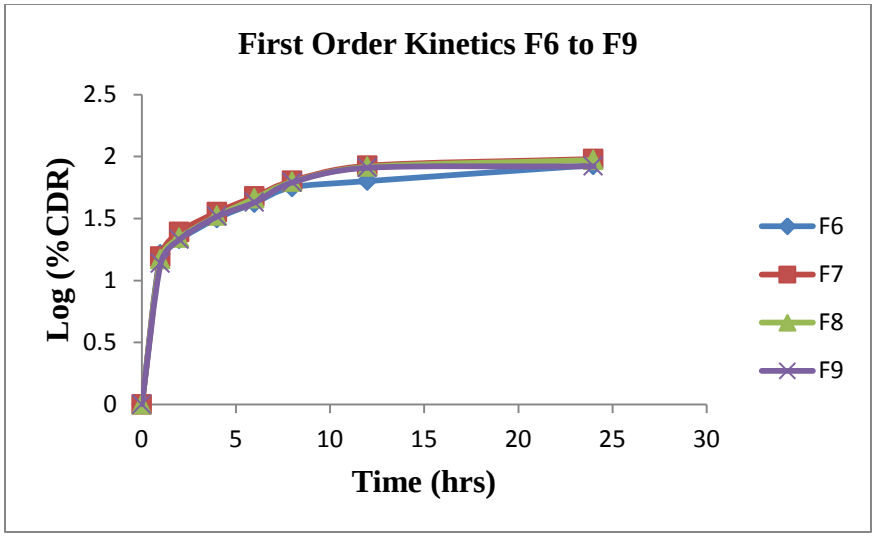

Figure 10: First order kinetics (F6-F9)

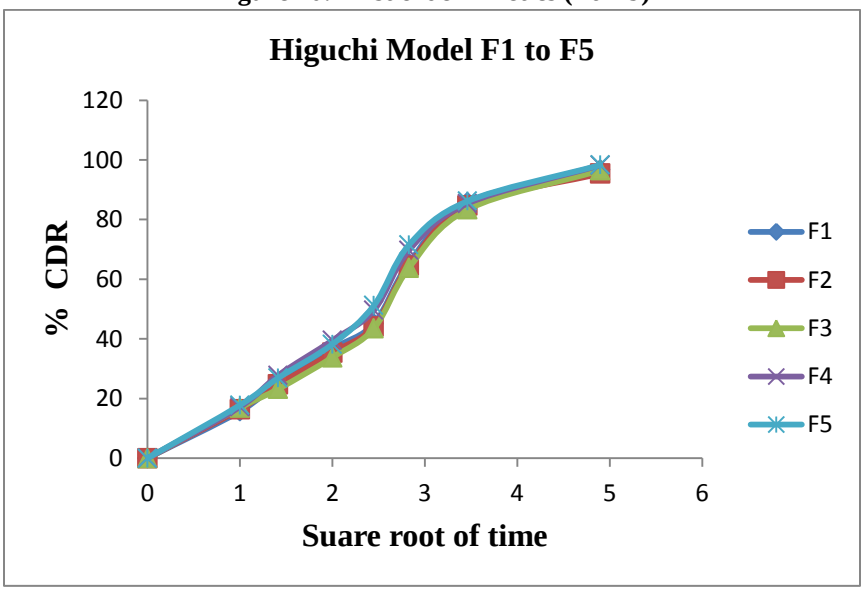

Figure 11: Higuchi model for the formulations F1-F5

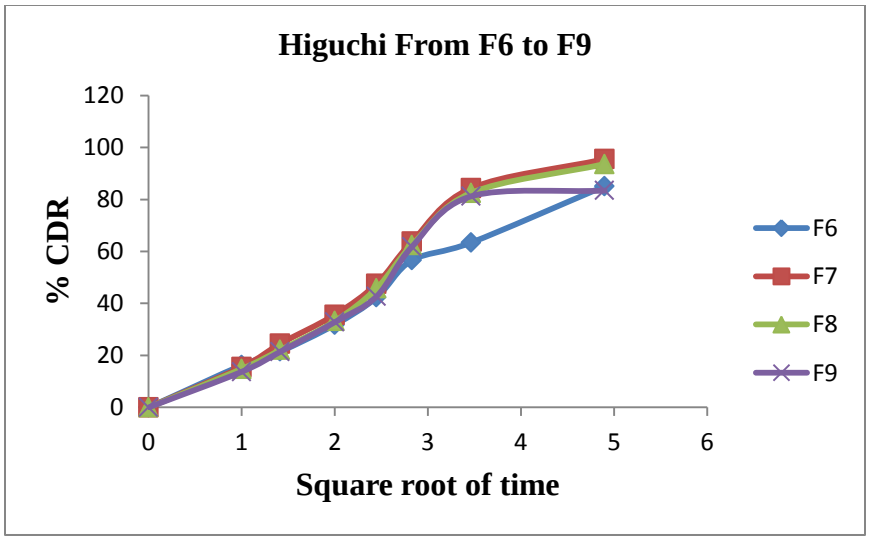

Figure 12: Higuchi model for the formulations F6-F9

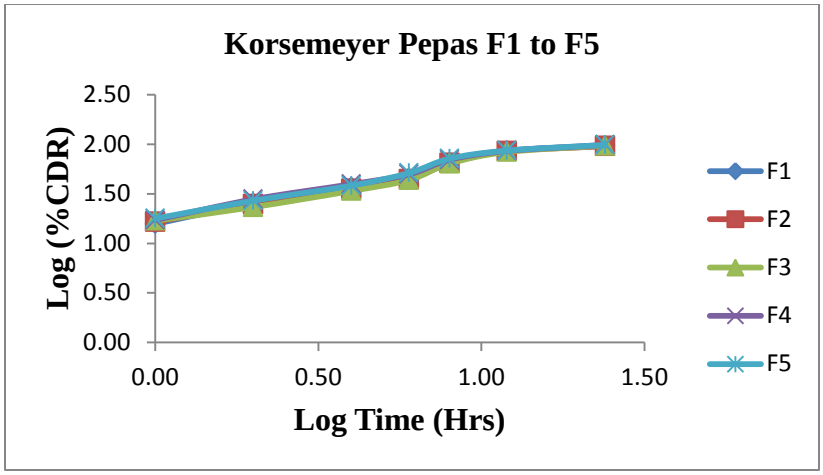

Figure 13: Korsemeyer Pepas plots for the formulations F1 to F5

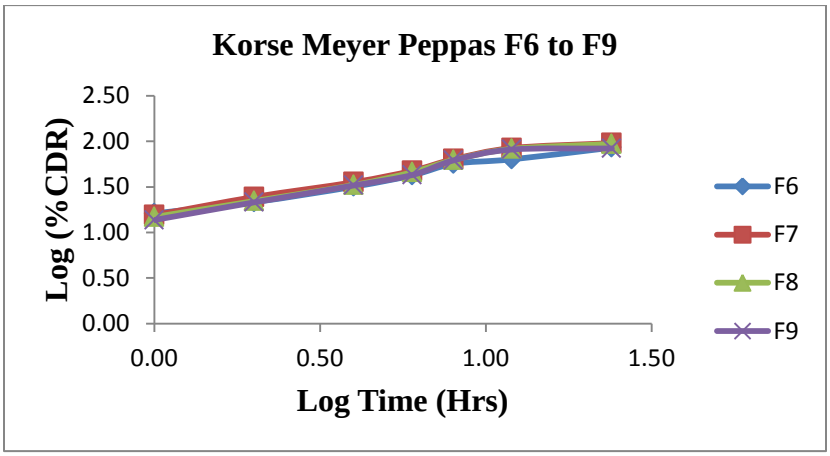

Figure 14: KorsemeyerPepas plots for the formulations F6 to F9

Table 5: Results of drug release data fitting to different models

Type of release	Parameter	Formulation Code								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero Order	R^2	0.838	0.839	0.855	0.825	0.817	0.874	0.839	0.843	0.783
First Order	R^2	0.431	0.431	0.440	0.410	0.410	0.428	0.433	0.446	0.434
Higuchi	R^2	0.957	0.956	0.96	0.964	0.958	0.986	0.962	0.958	0.927
KorsmeyerPeppas	R^2	0.973	0.972	0.971	0.974	0.971	0.985	0.977	0.975	0.959

Table 6: Drug release parameters for various trial formulations:

S. No	Factorial Amount (mg)		Rel_2 h (%)	Rel_{12} h (%)	Rel_{24} h (%)
	A	B			
1	95:5	20	25.4	85.8	96.7
2	95:5	15	24.7	84.7	95.4
3	95:5	10	23.2	83.4	96.5
4	85:15	20	27.8	85.4	98.2
5	85:15	15	26.9	86.2	98.3
6	85:15	10	21.5	63.4	85.1
7	75:25	20	24.5	84.3	95.5
8	75:25	15	22.1	82.5	93.6
9	75:25	10	21.4	81.2	83.4

Optimization results: mathematical modeling

The statistical equations as per mathematical modeling for dependent response variables are given as follows:

The polynomial equation for the 3^2 factorial design is given as follows:

$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2, b_{11}X_1^2 + b_{22}X_2^2$

(a) For 2 hrs drug release:

The finalized equation in terms of coded factors is given as follows:

% drug release 2hrs, $Y_1 = 24.16 - 0.0667X_1Y + 0.8333X_2Y + 1.375X_1X_2Y – 1.85X_1^2 - 0.6X_2^2$

(b) For 12 hrs drug release:

The finalized equation in terms of coded factors is given as follows:

% drug release 12 hrs, $Y_2 = 81.87 - 0.35X_1Y + 3.666X_2Y + 0.65X_1X_2Y + 5.316X_1^2 – 3.883X_2^2$

(c) For 24 hrs drug release:

The finalized equation in terms of coded factors is given as follows:

% drug release 24 hrs, $Y_3 = 93.63 - 0.48X_1Y + 1.9X_2Y + 1.4X_1X_2Y - 0.35X_1^2 – 3.2X_2^2$

The coefficients of X_1, Y_1, Y_2, Y_3 and Y_4 indicate the drug retardation upon increase in lipid concentration and can be altered upon selection of suitable lecithin: surfactant ratio. Further, the response surface plots indicate the effect of independent variables on the dependent variables which justify F5 as the optimized formulation.

CONCLUSION

The investigation envisions the effect of Lecithin: span 80 in producing the optimized formulation of Mianserintransferosomes with the aid of 3^2 factorial design. From the results it can be concluded that the rate of drug release is directly proportion al to the concentration of lipid. Further, from the evaluation studies of drug content, entrapment efficacy and vesicle size it can be justified that F5 is optimized in comparison to the remaining formulations. Although the drug content is more elevated for F4 and F3 and F6 and F9 exhibits more sustain release activity, the entrapment efficacy is enhanced for F5 which clarifies a dosage regimen of once a day. The reason for the entrapment efficacy of F4 and F3 is the concentration of surfactant which after a cetin concentration causes the leakage of the vesicles thereby declining the entrapment efficacy. In connection to the above, the optimized formulation follows Higuchi kinetics and the drug release mechanism follows first order release type. Therefore, the current formulation can be successfully used for the treatment of depression with enhanced patient compliance and reduced dosing frequency.

ACKNOWLEDGEMENTS

We strongly acknowledge the management of PRRM College of Pharmacy for their technical and financial support without which the current investigation could not have been completed.

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