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Optimization and Characterization of Transdermal Ethosomal Gel of Fluoxetine by Hot Method

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Abstract

The current exploration is to develop Transdermal Ethosomal Gel of Fluoxetine using hot method. The study incorporates 3^2 factorial design for optimization of lecithin and ethanol which are considered as independent variables and drug content, entrapment efficacy, and vesicular size as dependent variables. The in-vitro studies reveal that F8 with lecithin: ethanol ratio 200:3, drug content of 95.658%, and entrapment efficacy of 93.853% is considered as optimized. Further, ANOVA studies and polynomial equations reveal the effect of lecithin on dependent variables and strengthen the statistical data. Hence, we conclude that the prepared Ethosomal Gel meets the specified criteria.

Keywords: Fluoxetine, Transdermal, Ethosomes, Factorial design

1. Introduction

The vesicular drug delivery is gaining importance now days for successful drug delivery into the steep layers of the skin. Transdermal drug delivery is versatile and is gaining importance because of its non-invasiveness and inhibits enzymatic drug degradation following transdermal administration. Further, the complications such as gastric irritation, first pass metabolism associated with oral administration and discomforts associated with parenteral administration are successfully prevented[1-3]. Despite of several challenges, the transdermal drug delivery offers numerous advantages such as large and readily accessible surface area for adsorption, ease of application and simultaneously termination of therapy. Hence, several drug molecules are embedded in the form of ethosomes through the incorporation of numerous penetration enhancers and vesicular carriers[4]. Ethosomes are a part of the vesicular drug delivery system containing hydroalcoholic, or hydro/ alcoholic/ glycolic phospholipids consisting of elevated alcoholic concentrations. Ethosomes are preferred for numerous drug formulations as they offer specific properties such as liquidity and deformability. Furthermore, the ethosomes exhibit enhanced entrapment efficacy and permeability which attracts the formulators towards ethosomal drug delivery system. In addition, the

physiochemical properties of the ethosomes enable them to cross the stratum corneum of skin and enhance the drug bioavailability[5]. Hence, the current investigations are focused on the ethosomal drug delivery of Fluoxetine which is indicated for the treatment of major depressive disorder in adult patients and in pediatric patients aged 8 to 18 years. The pharmacokinetic data of Fluoxetine reveals that a single oral dose containing 40mg of Fluoxetine exhibits systemic bioavailability after 6 to 8 hrs and possess 94.5% protein binding. The declined free concentrations of Fluoxetine may not be sufficient for producing the required therapeutic activity and demands for increase in the dosage regimen[6-8]. Therefore, there arise a demand for developing a suitable formulation that can enhance the pharmacokinetic parameters and generates a sustain release of the drug. The drug can be delivered to the targeted site successfully through a careful selection of lipid: ethanol ratio. Because the lipid is preferred for the encapsulation of drug and exhibits a sustained release character and the ethanol plays a crucial role in developing the vesicle size and drug content. It is believed that the drug absorption occurs by ethanol effect and ethosomal effect. As discussed earlier the ethanol enhances the membrane fluidity and in turn decreases the density of lipid layers of cell membrane which makes the ethosomes to get fused with the lipid layers which encourages drug delivery into the deep layers of skin. The above criteria are fulfilled successfully through ethosomal gel formulation with the aid of response surface methodology. The design is generated through design expert 11® trial version software in which the effect of two independent variables i.e. lecithin and ethanol on various dependent variables such as entrapment efficacy, drug content are extensively studied[9-12]. Further, various coded equations for dependent variables are generated that are extensively used for studying the effect of dependent variables on independent variables. Apart from the above, various counter 2D plots are generated that serve as a means for enhancing the data predictability and for justification of the results[13-14]. On overall, ethosomes serve as a better means for transdermal drug delivery that owns a notable transdermal flux in comparison to the traditional liposomes.

1. Materials and Methods

1.1 Drug and Chemicals Used

Fluoxetine was procured from Yarrow Chemicals, Ahmedabad, India. Lecithin and Ethanol are procured from S.D fine Chemicals, Mumbai, India. Propylene glycol and Cholesterol are procured from Finar Chemicals, Mumbai India.

1.2 Preparation of Ethosomes

The Ethosomes are prepared by hot method in which lecithin is usually dissolved in water by heating the water bath at 40ºC until a colloidal solution is obtained. In another beaker, Fluoxetine is dissolved in ethanol and the specified quantity of propylene glycol is added and maintained at 40ºC. When the temperature of both the containers attains equilibrium, then the organic phase is mixed with the aqueous phase and subjected to sonification for generating the desired vesicles[15].

1.2.1 Construction of calibration curve

For the preparation of calibration curve 100mg of Fluoxetine is dissolved in 100ml of ethanol to obtain a concentration of 1000µg/ml (stock solution I) and from that nearly 10 ml of the solution is pipetted out and diluted with 100ml of ethanol to obtain a concentration of 100µg/ml (stock solution II). From the stock solution B, 1, 2, 3, 4, 6 and 5ml are withdrawn and diluted with 10mlof ethanol to get the final concentration to 10, 20, 30, 40, 50, and 60µg/ml respectively and the prepared

concentrations are subjected to UV spectroscopy at 216nm.

Table 1:Standard Curve for Fluoxetine

Concentration (µg/ml)	Absorbance
0	0
10	0.159
20	0.285
30	0.439
40	0.598
50	0.743
60	0.882

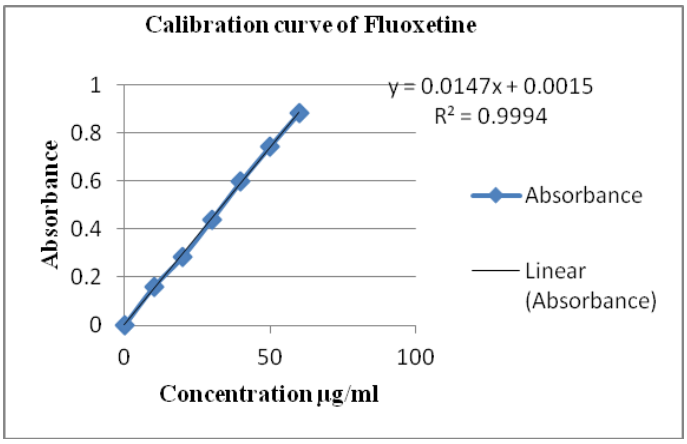


Table-2: Formulation chart of Fluoxetine Ethosomes

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Fluoxetine (mg)	10	10	10	10	10	10	10	10	10
Lecithin (mg)	100	100	100	150	150	150	200	200	200
Ethanol (ml)	2	3	4	2	3	4	2	3	4
Propylene Glycol (ml)	1	1	1	1	1	1	1	1	1
Cholesterol (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Water (ml)	10	10	10	10	10	10	10	10	10

2.2.2 Preparation of transdermal gel of Fluoxetine:

1% of Carbopol D934 is added to pure water and kept aside for 20min. To the current mixture10ml of the ethosomal suspension is incorporated and 0.05ml of Triethanolamine is added slowly drop by drop to adjust the pH of the preparation to 7.4. Therefore, the resultant is evaluated for viscosity, pH, in-vitro drug release study.

Table 3: Experimental design and statistical analysis

	Levels Used		
Independent Variables	**-1**	**0**	**+1**
A: lecithin (mg)	100	150	200
B: Ethanol (ml)	2	3	4
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 ($T_{50\%}$)		

2. Evaluation and Characterization of Ethosomes

2.1 Vesicle size analysis

The vesicle size analysis is performed using an optical microscope at 40X magnification. The size is measured using pre-calibrated ocular micrometer at 40X magnification and the process is continued for nearly 50 vesicles and the average values are evaluated for deciding the optimized formulation[16-18].

2.2 Shape and surface morphology

The shape and surface morphology of the prepared formulations were observed using capture pro 4.0® software at 10X magnification[19].

2.3 Drug Content

In the current investigation, drug content is assessed by taking 1ml of the formulation and sufficiently diluting it in the Beer's range between 1-12µg/ml and observing the absorbance values at 261nm[20]. Te obtained values are utilized for calculating the drug content using the following equation

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

2.4 Entrapment efficiency

The amount of drug entrapped in the given formulation is determined through ultra centrifugation technique in which a specified quantity of 2ml is placed in the ultracentrifuge and operated at 1200rpm for 20min. The resultant is subjected to decantation for the separation of supernatant liquid and analyzed for the determination of unentrapped drug through UV spectrophotometer at 261nm [21]. Further, the entrapment efficacy is calculated by using the following formula

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

In the above formula the amount of drug entrapped specifies to difference in concentration of Fluoxetine incorporated to the concentration assessed spectrophotometrically.

Table 4: Drug Content and Entrapment Efficiency

Formulation Code	Lecithin (mg)	Ethanol (ml)	Drug Content	Entrapment Efficacy (%)
1	200	2	93.457	90.782
2	150	2	94.245	85.892
3	200	4	94.685	92.542
4	100	2	93.897	82.751
5	100	4	94.234	85.698
6	100	3	94.257	87.564
7	100	3	93.821	87.685
8	200	3	94.658	93.853
9	100	2	94.213	82.983
10	200	4	93.204	92.654
11	150	2	94.864	85.785
12	150	4	94.521	87.569
13	200	3	93.283	93.254
14	150	3	94.285	89.325

The three square factorial design was generated Design Expert® 11 with 4 replicates that ultimately produced 14 formulations.

Table 5: Preparation of Fluoxetine Ethosomal Gel

Formulation Code	Carbopol D934 (%)	Ethosomal Suspension (ml)	Triethanolamine (ml)	Water
FG1	1	10	0.05	Q.S
FG2	1	10	0.05	Q.S
FG3	1	10	0.05	Q.S
FG4	1	10	0.05	Q.S

3.6 Determination of pH for Fluoxetine Ethosomal Gel

The pH of the prepared formulation is determined by using pH meter consisting of glass electrode. Initially the pH meter is calibrated by immersing it in buffer of pH 7.4 and following the above, it is immersed in ethosomal gel for the determination of pH[22].

3.7 Determination of Viscosity

The viscosity is determined by using Brookfield viscometer. 50gms of the prepared formulation is weighed and taken in a beaker. The brook field viscometer containing T-shaped spindle is completely immersed in the specified formulation and allowed to move up and down at various points in the formulation[23]. The viscosity thus determined at various points is averaged and the viscosity of formulation is evaluated.

3.8 In-vitro Drug release Analysis

The in-vitro drug release studies are performed using Franz diffusion studies which consists of egg membrane mounted in an upright position into the donor compartment. The recipient compartment consists of 250ml phosphate buffer pH 7.4. About 1gm of the prepared formulation is placed on the donor compartment and operated at 50rpm for 24 hrs at 37±5ºC. Simultaneously the solution in the recipient compartment is stirred at 50rpm using a magnetic stirrer and meanwhile, 5ml of the sample is withdraw at pre-requisite time intervals and the same was replaced with fresh phosphate buffer pH 7.4 for maintaining the simulated conditions[24-25]. The withdrawn samples are analyzed for drug content at 216 nm using UV spectrophotometer.

3.9 Release kinetics

The mechanism of drug release from generated formulations can be explored by employing linear regression analysis to the in-vitro drug release data through MS Excel[26]. The graphical data is used for

obtaining the regression coefficient values and "n" values which on intense differentiation with the standard values reveal the type of mechanism and transport invoved.

4 Results and Discussion

4.1 Vesicle Size Analysis

The results predicted in table 9 for vesicle size details that the vesicle size increased with the lecithin concentration and the same decreases with the increase in ethanol concentration. The theory behind the above prediction is due to impact of ethanol that altered the net charge and conferred to steric stabilization that reduced the vesicle size. In the obtained results F8 and F13 containing the same composition exhibited identical vesicular size which is due to the enhancement in lecithin concentration and F3 and F10 reveals a declination in vesicles size due to the effect of ethanol concentration.

4.1 Shape and surface morphology

The surface and morphological characteristics were studied using Capture pro 4.0 ® software which reveals that the generated vesicles were uniform, small and unilamellar in size.

4.2 Drug Content and Entrapment efficiency

The results of drug content and entrapment efficacy are depicted in table 4 which confirms that F11 has the highest drug content of 94.864% and must be declared as the best formulation. But the entrapment efficacy of the same exhibits 85.785% and rather than this F8 contains the highest entrapment efficacy of 93.853% which might be due to variation in the lecithin: ethanol ratio and their effect on the formulation with increasing concentration. It is hypothesized that elevated levels of ethanol concentration creates modification in the net charge, steric stabilization and causes fluidization of the membrane, thereby enhancing the entrapment efficacy. Further the higher ethanol concentration causes the membrane more leaky and thereby decreasing the entrapment efficacy. Since the optimization is based on the entrapment efficacy, the formulation F8 consisting of 200mgof lecithin and 3ml of ethanol is considered as optimized and fit for commercial development. In continuation to the above, the coded regression equations developed for the dependent variables using Design Expert 11.0® software reveals the effect of lecithin and ethanol on the independent variables. The equations depicted below justify A as the lecithin concentration and B as the ethanol concentration and AB as the interaction of both and their corresponding effect on the independent variables. Hence, from the equations it can be conferred that the positive sign as increase in the enhancement in entrapment efficacy and drug content while the negative sign it's vice versa. Further, the above theory can be correlated through Counter diagrams and 3D plots depicted against variable concentrations of lecithin and ethanol.

Coded equations for various dependent variables is depicted as follows

$$\text{Entrapment efficacy} = 89.33 + 2.96\,A + 0.8653B - 0.2537AB + 1.26A^2 - 2.62B^2 + 0.2965A^2B + 0.7392\,AB^2 - 0.0185\,A^2B^2$$

$$\text{Drug Content} = 94.29 + 0.3095A - 0.0168B - 0.1475AB + 0.0635A^2 + 0.2528B^2 + 0.0377A2B - 0.6770\,AB^2 - 0.9032\,A^2B^2$$

Table 6: In-vitro Drug release Study

Time (hrs)	% Cumulative Drug Release													
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
1	16.7	15.4	15.9	18.2	19.8	17.2	18.5	15.8	17.7	16.9	16.4	18.6	15.4	17.6
2	27.4	25.7	28.2	29.8	30.9	29.5	31.5	28.1	27.4	29.2	23.7	31.2	22.8	28.5
4	38.7	39.4	39.8	42.5	46.2	44.8	43.6	39.2	42.8	38.2	37.4	44.3	36.4	41.2
6	47.1	48.2	48.5	54.6	58.2	56.3	55.5	48.8	55.1	46.5	47.2	55.1	45.2	52.4
8	62.4	64.5	72.8	74.7	79.5	77.6	76.7	64.4	75.8	71.8	63.5	75.4	61.5	73.1
12	75.8	79.7	85.4	88.4	90.2	89.4	88.3	77.5	87.2	84.4	78.7	89.2	76.1	86.3
24	85.3	89.4	91.2	94.2	98.3	96.5	95.8	87.1	93.8	90.3	88.8	95.2	87.3	93.5

Table 7: In-vitro drug release studies at various time interval.

Formulation	Factorial Amount (mg)		Rel_2 h (%)	Rel_{12} h (%)	Rel_{24} h (%)
	A	B			
F1	200	2	27.4	75.8	85.3
F2	150	2	25.7	79.7	89.4
F3	200	4	28.2	85.4	91.2
F4	100	2	29.8	88.4	94.2
F5	100	4	30.9	90.2	98.3
F6	100	3	29.5	89.4	96.5
F7	100	3	31.5	88.3	95.8
F8	200	3	28.1	77.5	87.1
F9	100	2	27.4	87.2	93.8
F10	200	4	29.2	84.4	90.3
F11	150	2	23.7	78.7	88.8
F12	150	4	31.2	89.2	95.2
F13	200	3	22.8	76.1	86.3
F14	150	3	28.5	87.3	93.5

The in-vitro drug release studies are reveled in table which depicts an inverse proportionality relationship between the lecithin concentration and % drug release. It is believed that as the concentration of lecithin increases the % drug release decreases simultaneously. But the design depicts a simultaneous increase in the ethanol concentration with respect to lecithin concentration which exhibits a profound effect on the percentage drug release of Fluoxetine. This is because the ethanol has the property of membrane fluidization effect and can cause the membrane more leaky when certain concentration exceeds which is against to the sustain release effect. The current theory can be correlated to the above results in which F5 and F6 competes for the % drug release containing 100mg of drug and exhibits 98.3% and 96.5% release respectively. Apart from this, F1 containing 200mg of Fluoxetine and 2ml of ethanol exhibits 85.3% drug release, F3 containing 200mg of Fluoxetine and 4ml of ethanol exhibits 91.2 % drug release, and F8 containing 200mg of Fluoxetine and 3ml of ethanol exhibits 87.1 % drug release. Hence, as per the ongoing discussion F1 should be considered as the optimized formulation, but instead F8 is highlighted because of the enhanced entrapment efficacy, drug content and negotiable difference in % drug release between F1and F8. Further, the polynomial equations in terms of coded variables for % drug release for 2hrs, 12hrs and 24hrs are developed using Design expert® 11.0 software which reveals the effect of lecithin and ethanol on the % drug release. In the equations the positive sign is considered as the enhancement in % drug release due to the effect of independent variable and the negative sign indicates it's vice versa. The polynomial equations in terms of coded factors are represented as follows:

$$Rel_2\ h\ (\%) = 28.50 - 1.70A + 3.25B + 0.1750AB + 1.30A^2 - 0.5500B^2 - 2.53A^2B + 0.6750AB^2 + 0.0750A^2B^2$$

$$Rel_{12}\ h\ (\%) = 87.30 - 5.40A + 5.00B + 1.70AB - 4.40A^2 - 3.10B^2 - 2.40A^2B + 0.80000AB^2 + 4.90A^2B^2$$

$$Rel_{24}\ h\ (\%) = 93.50 - 4.35A + 3.05B + 0.2250AB - 2.05A^2 - 1.35B^2 - 0.7750A^2B + 0.1250AB^2 + 1.93A^2B^2$$

4.3 Kinetic studies

Table 8: Kinetic parameters for various formulations

Formulation Code	Kinetic Parameters			
	Zero Order	First Order	Higuchi	Korsemeyer peppas
	Regression Coefficient	Regression Coefficient	Regression Coefficient	Regression Coefficient
F1	0.821	0.389	0.962	0.972
F2	0.818	0.407	0.960	0.970
F3	0.765	0.398	0.932	0.951
F4	0.765	0.382	0.937	0.957
F5	0.763	0.373	0.938	0.958
F6	0.757	0.385	0.936	0.952
F7	0.768	0.377	0.939	0.956
F8	0.811	0.392	0.959	0.965
F9	0.756	0.388	0.934	0.955
F10	0.772	0.394	0.932	0.950
F11	0.827	0.412	0.961	0.974
F12	0.766	0.376	0.939	0.957
F13	0.837	0.421	0.964	0.975
F14	0.779	0.390	0.942	0.961

Figure 1

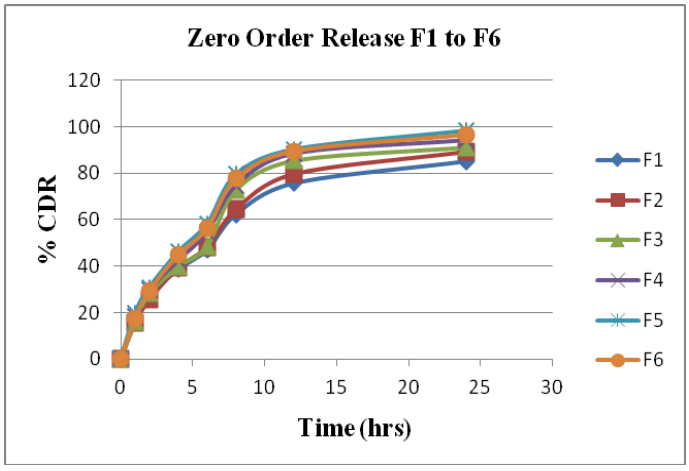


Figure 2

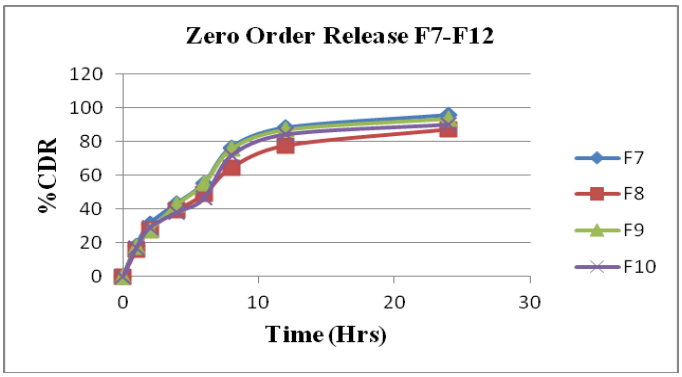


Figure 3

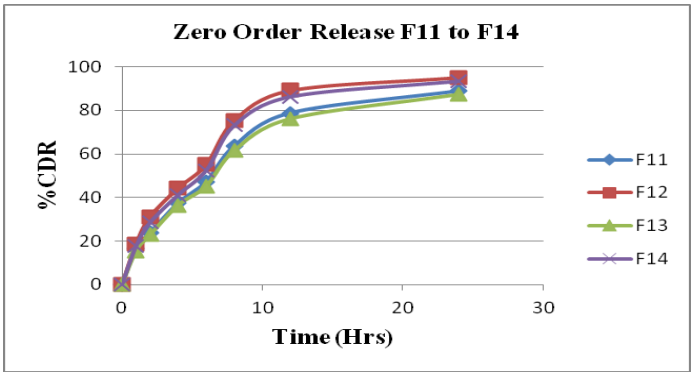
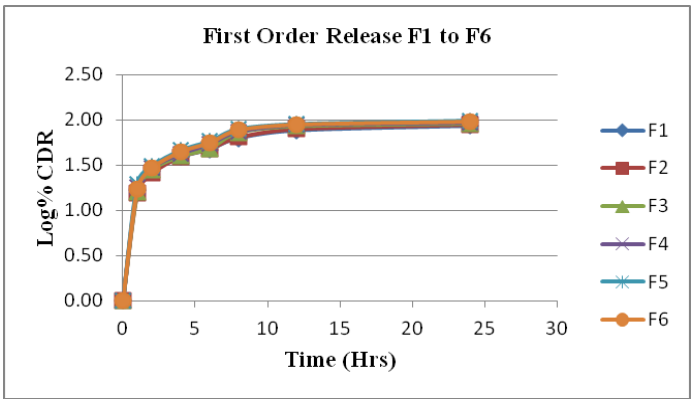


Figure 4



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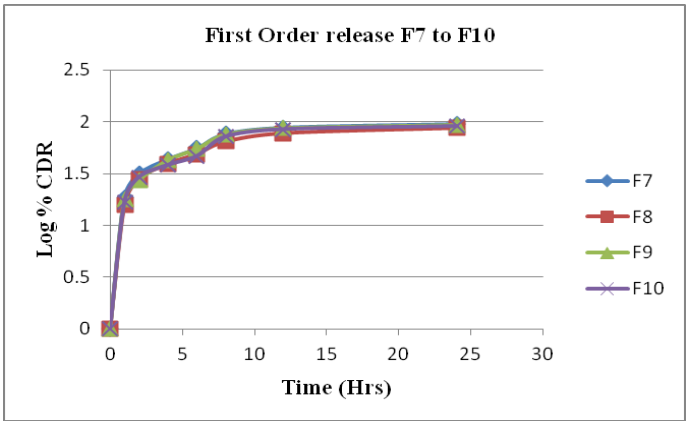

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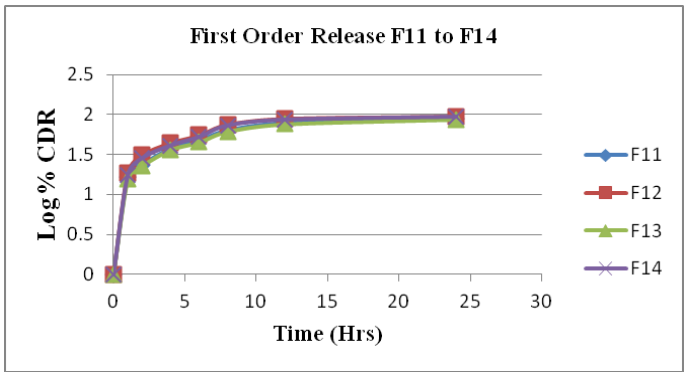

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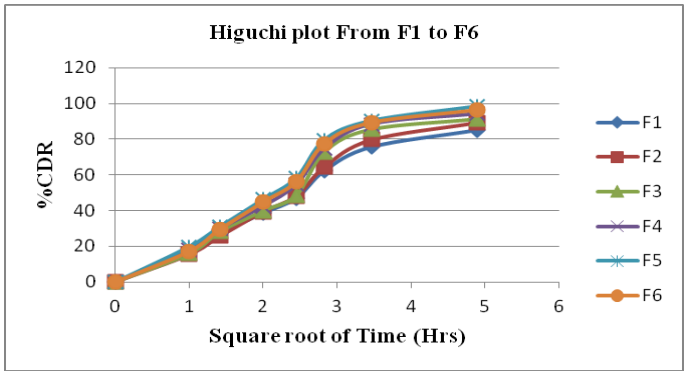

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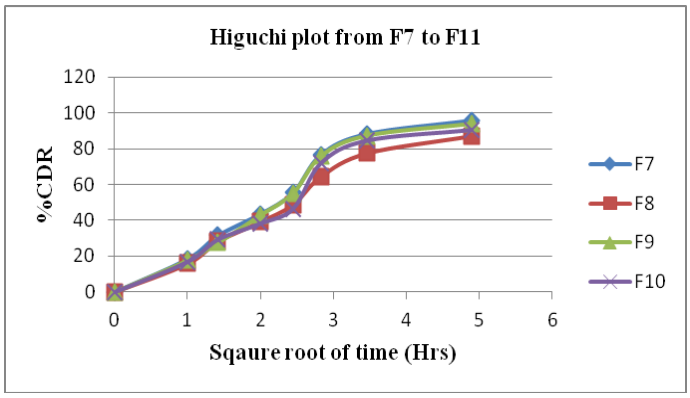


Figure 9

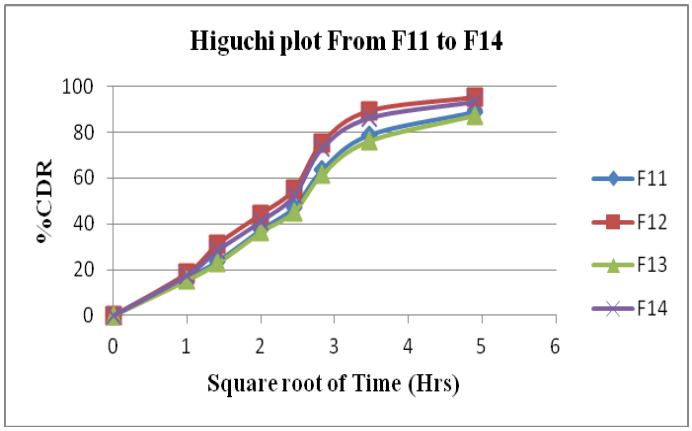


Figure 10

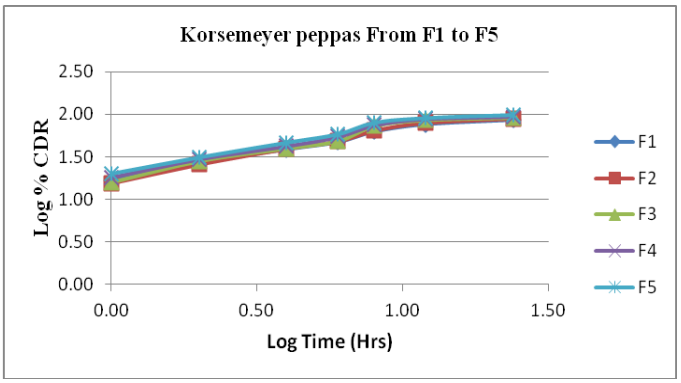


Figure 11

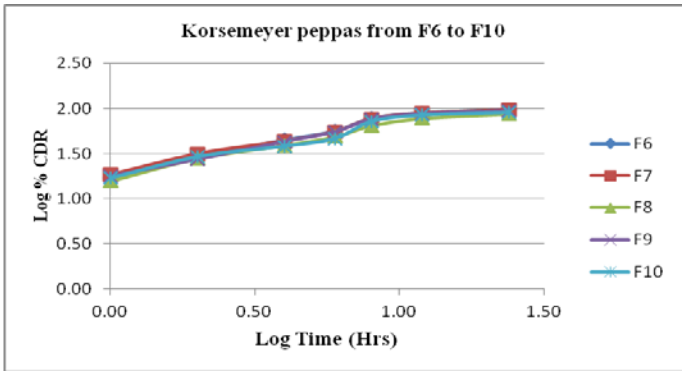
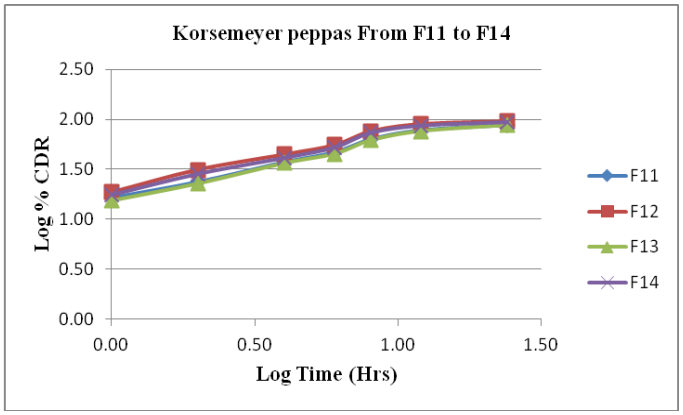


Figure 12



4.6 pH and Viscosity Analysis

The pH and Viscosity analysis is performed using glass electrode and Brook field viscometer as mentioned above. The obtained results are depicted in table 9 which disclose that they are within pharmacopoeial limits and the prepared formulations are devoid of skin irritations and are worthy for transdermal application.

Table 9 comparison of pH, viscosity, and vesicle size from F1-F14

Formulation Code	pH	Viscosity	Vesicle size
F1	6.86	3546	12.47
F2	9.54	3875	9.87
F3	6.83	3684	13.54
F4	6.58	3758	6.35
F5	6.62	3652	7.54
F6	6.48	3942	8.72
F7	6.75	3852	8.84
F8	6.27	3749	15.21
F9	6.38	3853	6.29
F10	6.41	3957	13.68
F11	6.28	3759	9.86
F12	6.75	3861	10.76
F13	6.53	3794	15.34
F14	6.27	3243	11.89

ANOVA for Reduced Quartic model

Model	178.40	8	22.30	494.17	< 0.0001	significant
A-Lecithin	35.15	1	35.15	779.00	< 0.0001	
B-Ethanol	2.00	1	2.00	44.24	0.0012	
AB	0.3434	1	0.3434	7.61	0.0399	
A^2	1.28	1	1.28	28.32	0.0031	
B^2	5.00	1	5.00	110.74	0.0001	
A^2B	0.1563	1	0.1563	3.46	0.1218	
AB^2	1.25	1	1.25	27.68	0.0033	
A^2B^2	0.0002	1	0.0002	0.0042	0.9509	
Pure Error	0.2256	5	0.0451			
Cor Total	178.62	13				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The Model F-value of 494.17 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2, B^2, AB^2 are significant model terms.

Response 1: Entrapment Efficacy

Std. Dev.	0.2124	**R^2**	0.9987
Mean	88.45	**Adjusted R^2**	0.9967
C.V. %	0.2402	**Predicted R^2**	NA
		Adeq Precision	62.7430

ANOVA for Reduced Quartic model

Response 1: Drug Content

Model	3.37	8	0.4214	7.35	0.0209	significant
A-Lecithin	0.3832	1	0.3832	6.68	0.0491	
B-Ethanol	0.0007	1	0.0007	0.0131	0.9135	
AB	0.1160	1	0.1160	2.02	0.2141	
A^2	0.0032	1	0.0032	0.0563	0.8219	
B^2	0.0465	1	0.0465	0.8105	0.4092	
A^2B	0.0025	1	0.0025	0.0442	0.8418	
AB^2	1.05	1	1.05	18.27	0.0079	
A^2B^2	0.4501	1	0.4501	7.85	0.0379	
Pure Error	0.2866	5	0.0573			
Cor Total	3.66	13				

Fit Statistics

Std. Dev.	0.2394	**R^2**	0.9216
Mean	94.09	**Adjusted R^2**	0.7963
C.V. %	0.2545	**Predicted R^2**	NA
		Adeq Precision	7.5741

Response 1: Rel2 h

Model	65.12	8	8.14	20.35	0.0021	significant
A-Lecithin	7.71	1	7.71	19.27	0.0071	
B-Ethanol	28.17	1	28.17	70.42	0.0004	
AB	0.1633	1	0.1633	0.4083	0.5509	
A^2	1.93	1	1.93	4.83	0.0794	
B^2	0.3457	1	0.3457	0.8643	0.3952	
A^2B	11.33	1	11.33	28.34	0.0031	
AB^2	0.8100	1	0.8100	2.02	0.2140	
A^2B^2	0.0039	1	0.0039	0.0098	0.9251	
Pure Error	2.00	5	0.4000			
Cor Total	67.12	13				

Fit Statistics

Std. Dev.	*0.6325*	***R^2***	*0.9702*
Mean	*28.80*	***Adjusted R^2***	*0.9225*
C.V. %	*2.20*	***Predicted R^2***	*NA*
		Adeq Precision	*13.4098*

Response 2: Rel12 h

Model	385.15	8	48.14	481.44	< 0.0001	Significant
A-Lecithin	77.76	1	77.76	777.60	< 0.0001	
B-Ethanol	66.67	1	66.67	666.67	< 0.0001	
AB	15.41	1	15.41	154.13	< 0.0001	
A^2	22.13	1	22.13	221.26	< 0.0001	
B^2	10.98	1	10.98	109.83	0.0001	
A^2B	10.24	1	10.24	102.40	0.0002	
AB^2	1.14	1	1.14	11.38	0.0198	
A^2B^2	16.70	1	16.70	167.03	< 0.0001	
Pure Error	0.5000	5	0.1000			

Fit Statistics

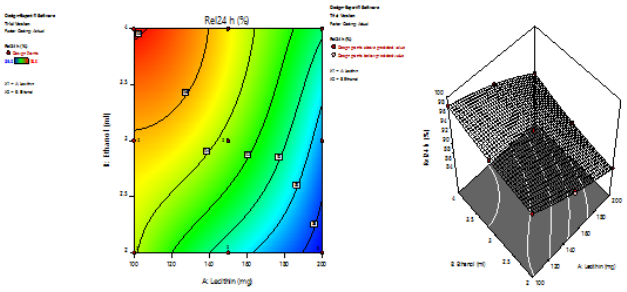
Std. Dev.	0.3162	R^2	0.9987
Mean	84.24	Adjusted R^2	0.9966
C.V. %	0.3754	Predicted R^2	NA
		Adeq Precision	56.7944

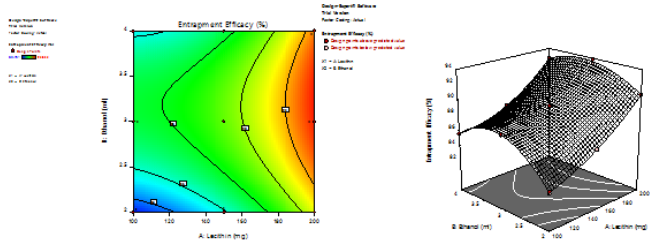
Response 3: Rel24 h

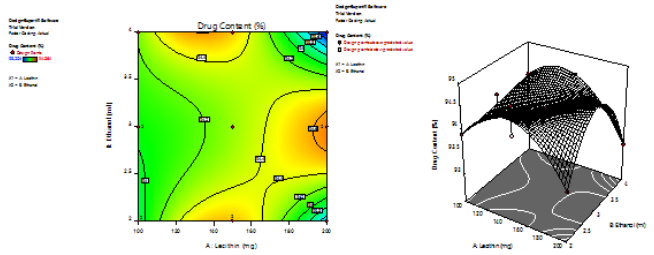
Model	226.37	8	28.30	786.00	< 0.0001	significant
A-Lecithin	50.46	1	50.46	1401.67	< 0.0001	
B-Ethanol	24.81	1	24.81	689.07	< 0.0001	
AB	0.2700	1	0.2700	7.50	0.0409	
A^2	4.80	1	4.80	133.41	< 0.0001	
B^2	2.08	1	2.08	57.86	0.0006	
A^2B	1.07	1	1.07	29.66	0.0028	
AB^2	0.0278	1	0.0278	0.7716	0.4199	
A^2B^2	2.58	1	2.58	71.61	0.0004	
Pure Error	0.1800	5	0.0360			
Cor Total	226.55	13				

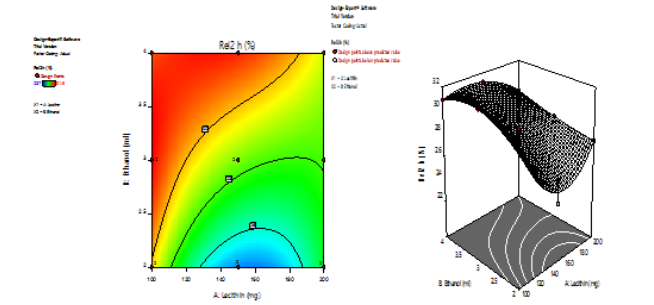
Fit Statistics

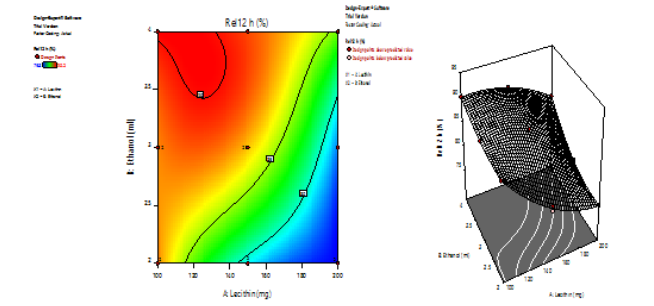
Std. Dev.	0.1897	**R^2**	0.9992
Mean	91.91	**Adjusted R^2**	0.9979
C.V. %	0.2064	**Predicted R^2**	NA
		Adeq Precision	85.4545











6. Conclusion and Future scope

The Present Research is carried out to trace out the optimized Transdermal Gel formulation of Fluoxetine using hot method. As a part of the investigation, we preferred 3^2 factorial design for optimization using design expert 11.0® software (trial version). The design generated total 9 formulations with varied concentrations of lecithin and cholesterol and the generated results are subjected to statistical analysis such as ANOVA for tracing out the optimized formulation. Therefore, from the results we found F8 as quite optimized and meet the pharmaceutical and pharmacokinetic criteria. Further, the future scope of investigation relates to development of niosomes as promising carriers for delivery of various drug molecules with predetermined quality attributes.

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