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DESIGN AND CHARACTERIZATION OF TRANSDERMAL ETHOSOMAL GEL OF PAROXETINE BY COLD METHOD

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ABSTRACT

The present investigative work is carried out to formulate and optimize the Paroxetine ethosomal gel using cold method. As a part of the investigation, we used three square design using design expert® 11.0 version software for optimization of individual concentrations of lecithin and ethanol and are considered as the independent variables. Whereas, drug content, entrapment efficacy, and vesicular size are considered as dependent variables which gets altered due to independent variable concentrations. The study was carried out using 14 formulations containing the variable concentrations of lecithin and ethanol. Further, the in-vitro studies of the formulations reveal that F3 having lecithin: ethanol ratio as 100:5 and exhibiting the drug content of 95.285% and entrapment efficacy of 95.354% is considered as optimized. In continuation to the above, F3 exhibits first order release (R^2 = 0.951) mechanism with non- Fickian diffusion. The ANOVA studies and polynomial equations in terms of coded equations were generated for the obtained results which reveal the effect of lecithin on the dependent variables and strengthen the statistical data. On the whole, it can be concluded that F3 is optimized and can serve as an effective carrier for transdermal drug delivery of Paroxetine in generating the required therapeutic effect.

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INTRODUCTION

Despite of the investigations going in the dermal and transdermal drug delivery, the topical administration of drugs remains a challenge. Among the various routes of drug administration, transdermal drug delivery exhibits a promising result when compared to the oral route of administratin.[1-3] The crucial factors that enable the transdermal drug delivery a choice for drug transport are its gastrointestinal encompassing and inhibition of first pass metabolism. Apart from the above, it also suffers with few drawbacks such as barrier properties of skin that enable only lipoidal moieties having molecular weight of less than 500daltons can pass through. Therefore, to overcome the existing the difficulty several mechanisms were investigated which include iontophoresis, sonophoresis, et Along with these; there are vesicular drug delivery systems such as niosomes, transferosomes, pharmacosomes etc that can deliver the drug across the skin through the aid of permeation enhancers. Even though there are permeation enhancers, the extent of permeation for many vesicular drug delivery systems are limited to the outer most layers of the skin. Therefore, the investigations were carried out to trace out a suitable drug delivery system that can effectively transport the drug molecules to the innermost layers of the skin[4-5]. As a result the ethosomes were developed which are specified as "ethanolic liposomes" and can be defined as the non-invasive drug delivery carriers that can enable the drug to reach the deep layers of the skin / or into the systemic circulation[6]. The vesicles prevent the drug molecules being captured by opsonins and enable controlled release of drug moieties. Further, the ethosomes are considered as the second generation of elastic liposomes containing a predefined concentration of ethanol and phospholipids in association with water. The size of ethosomes may vary from several tens of nanometers to the microns and possess transdermal flux which enables these to increase their surface area thereby increasing the dissolution characteristics. Apart from the above, the specific characteristics of ethosomes such as non-toxicity, capability to deliver large molecules, non-invasiveness, enhanced penetration, makes these a choice for targeted drug delivery[7-10]. In connection to the above, the ethosomes offer a better stability than the liposomes which to grow in size upon prolonged storage which generates drug leakage through the vesicles. Whereas, the ethosomes exhibit steric stabilization which can prevents agglomeration and drug leakage through vesicles. Therefore, the primary objective of the current investigation is focused on the development of ethosomal gel formulation of Paroxetine for delivering the drug moieties to the targeted site and to generate a sustained release effect. In connection to Paroxetine, it is BCS class II drug and hydrochloride salt of phenylpiperidine compound intended for the treatment of major depressive disorder, social anxiety disorder, obsessive compulsory disorder, panic disorder, generalized anxiety disorder, and post traumatic stress disorder. The mechanism of action revels that the Paroxetine potentiates serotonergic activity in CNS which ultimately results serotonin reuptake inhibition. The pharmacokinetic data reveals that the drug has a mean elimination half life of 21hours and exhibits 95% plasma protein binding. Therefore, enough free concentrations are not available for producing the required therapeutic effect. Further, the situation has made the investigators to think for an effective formulation that can enhance bioavailability and generate the required therapeutic effect[10-12]. The efficacy of ethosomal formulation is depending on the suitable ratio of lipid: ethanol which can be optimized through response surface methodology (RSM) techniques. The present investigation prefers three square factorial design using design expert® 11.0 software (trial version) for the optimization of independent variables such as lecithin and ethanol on various dependent variables such as vesicle size, drug content, and entrapment efficacy. Further, the generated results are subjected for ANOVA studies which generate coded equations for various dependent variables and are quite helpful for the data analysis and interpretation of the results[13-15].

MATERIALS AND METHODS

Drug and Chemicals Used

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

Preparation if Ethosomes

The Ethosomes are prepared by Cold method in which lecithin and Paroxetine are usually dissolved in water maintained at room temperature by vigorous stirring. To the above propylene glycol is added at a temperature of 30ºC. In a separate vessel water is heated to a temperature of 30ºC and is incorporated to the above mixture with stirring[16]. The stirring is carried out for 5min at 700rpm in a closed vessel for the production of Ethosomes. Further, the resultant can be subjected to either sonification or extrusion for reducing its size and can be stored in a refrigerator.

Table 1: Formulation chart of Paroxetine Ethosomes.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
Paroxetine (mg)	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Lecithin (mg)	100	100	100	150	150	150	200	200	200	150	150	100	100	100
Ethanol (ml)	3	4	5	3	4	5	3	4	5	3	4	5	5	3
Propylene Glycol (ml)	1	1	1	1	1	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	0.1	0.1	0.1	0.1	0.1
Water (ml)	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Preparation of transdermal gel of Paroxetine:

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. The resultant is evaluated for viscosity, pH, and in-vitro drug release study[17].

Table 2: Experimental design and statistical analysis.

	Levels Used		
Independent Variables	**-1**	**0**	**+1**
A: lecithin (mg)	100	150	200
B: Ethanol (ml)	3	4	5
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 ($T_{50\%}$)		

Evaluation and Characterization of Ethosomes

Vesicle size analysis

The vesicle size analysis is performed using optical microscopy at 40X magnification which includes observation of the vesicle size for nearly 25 vesicles for each batch size and the average values is determined[18-20].

Shape and surface morphology

The surface and morphological characteristics of Ethosomes are predicted using Capture Pro 4.0 ® software at 10Xmagnification[21].

Drug Content

The drug content is assessed by taking 1ml of the prepared formulation and diluting it in the beer's range of 2-10µg/ml and determining the absorbance at 294nm. Finally, the absorbance values are used for determining the drug content as follows[22-23]

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

Entrapment efficiency

The entrapment efficacy is determined by using ultra centrifugation in which 2ml of the prepared formulation is placed in the ultra centrifuge and operated at 1200 rpm for 20min. The supernatant liquid is separated by decantation and analyzed for unentrapped drug by UV spectrophotometer at 294nm. The entrapment efficacy is calculated by using the following formula[24-28]

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

Where amount of drug entrapped is difference between concentrations of paroxetine added initially to the concentration determined in the filtrate through spectrophotometrically.

Table 3: Entrapment efficiency.

Run	Lecithin (mg)	Ethanol (ml)	Entrapment Efficacy (%)	Drug Content (%)
1	150	4	87.623	94.32
2	200	5	91.842	94.352
3	200	4	94.354	95.285
4	150	3	84.625	94.368
5	100	3	81.736	94.542
6	200	3	90.452	94.326
7	100	4	83.679	94.254
8	150	5	85.824	94.561
9	200	4	94.142	95.324
10	150	3	84.564	94.352
11	150	4	87.542	93.875
12	100	5	82.621	94.567
13	100	5	82.321	94.592
14	100	3	81.762	94.382

Table 4: Preparation of Paroxetine 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

Determination of pH for Paroxetine Ethosomes

The current study is carried out using a pH meter by dipping the glass electrode entirely in the prepared gel formulation and the corresponding readings are noted down. (Table 8)

Determination of Viscosity

The viscosity is determined by using Brookfield viscometer containing a T-bar spindle which measures the viscosity of the formulation. In general, the viscosity is determined by dipping the spindle in a 100ml beaker containing 50gm of the prepared formulation[29]. The instrument is lowered in such a way that the spindle does not touch the bottom of the container and the spindle is moved up and down to determine the viscosities of the preparations at various points. The obtained results are averaged to assess the viscosity of the formulation.

In-vitro Drug release Analysis

The in-vitro drug release studies are performed using Franz diffusion cell in which the treated egg membrane is mounted in an upright position into the donor compartment. Nearly 1gm of the gel is placed in the donor compartment which is enveloped with petroleum jelly and driven by using magnetic stirrer at 50rpm. The recipient compartment consists of phosphate buffer pH 7.4 at 37±5ºC and the study is carried out by taking 5ml of sample solution from the recipient compartment and the same being replaced with fresh buffered solution pH 7.4. The withdrawn sample is analyzed for drug concentration at 294nm using UV spectrophotometer[30-31].

Release Kinetics

The drug release mechanism can be clearly understood by subjecting the in-vitro drug release data to linear regression analysis through MS excel statistical functioning. As a part of analysis several graphical representation are predicted between % drug release and time which is in turn used for obtaining regression coefficient and "n" values. The obtained values on differentiation with the standard values predict the type of release and transport involved in it[32-35].

RESULTS AND DISCUSSION

Vesicle Size Analysis

The vesicle size is determined using optical microscopy technique and the results obtained were predicted in the table 8 which reveals that the vesicular size increases with the lecithin concentration up to a certain limit and further increase in the lecithin concentration exhibits a decrease in the vesicle size. The reason is due to the elevated levels of ethanol concentration which causes an alteration in the net charge and exhibits steric stabilization which results decrease in vesicular size.

Shape and surface morphology

The shape and surface morphology of the prepared ethosomes were studied using Capture Pro 4.0 software ® which reveals that the prepared vesicles are small unilamellar sized.

Drug Content and Entrapment efficiency

The current study involves development of various formulations using three square factorial design at 4 replicates by the aid of Design expert 11.0 ®software. The results generated for drug content and entrapment efficacy reveal that F3 and F9 has the highest drug content of 94.354% and 94.142% and entrapment efficacy of 92.85% and 95.324% and must be considered as optimized. The keen observation on the obtained result and in connection to the above theory, F2 should be considered as the optimized. But there is a decline in the values of both drug content and entrapment efficacy which might be due to the effect of ethanol concentration. It reveals that upon increase in lecithin and ethanol concentration, the drug content and entrapment efficacy also increases which is due to the membrane fluidization that causes the enhancement in entrapment efficacy. Further, extreme concentrations of ethanol generate peculiar properties such as net charge, steric stabilization which are responsible for the decrease in the above mentioned properties. Therefore, F3 having drug content of 95.285% and entrapment efficacy of 95.354% is considered as optimized. Apart from the above, the regression equations in terms of coded factors is generated using Design expert 11.0 ® software which specify A for lecithin concentration and B for ethanol concentration and AB for their interaction parameters and their effect on the dependent variables. The equations consist of positive signs for independent variables which signify that upon increase in their concentrations, there will be a simultaneous increase in the dependent variables and vice versa for negative sign. In order to better understand the effect of independent variable concentrations on dependent variables, counter plots and 3D plots are depicted which even serves as a means for better optimization within the specified limits. Further, The polynomial Equation in terms of coded factors for various dependent variables such as drug content and entrapment efficacy are represented as follows

$$\text{Drug Content} = 94.10 + 0.5252A + 0.1005B - 0.0229AB + 0.6818A^2 + 0.3630B^2 - 0.0646A^2B - 0.6161\,AB^2 - 0.7124A^2B^2$$

$$\text{Entrapment Efficacy: } 87.58 + 5.34A + 0.6148B + 0.1670\,AB + 1.43A^2 - 2.37B^2 - 0.0868A^2B - 0.8190AB^2 - 0.0147A^2B^2$$

Table 5: In-vitro Drug release Study.

Time	% Cumulative Drug Release													
(hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
1	14.7	15.4	15.9	16.2	17.8	15.2	15.5	14.8	13.7	17.2	15.7	16.5	14.4	16.4
2	26.4	23.7	22.2	26.8	26.9	21.5	24.5	22.1	21.4	27.8	25.4	25.5	27.7	24.7
4	35.7	36.4	32.8	38.5	38.2	31.8	35.6	33.2	32.8	39.5	36.7	36.6	38.4	36.4
6	42.1	43.2	42.5	48.6	51.2	42.3	47.5	45.8	42.7	49.6	45.1	48.5	45.2	47.2
8	64.4	65.5	62.8	68.7	71.5	56.6	63.7	62.4	61.8	69.7	65.4	64.7	72.5	64.5
12	87.8	73.7	72.4	84.4	86.2	63.4	84.3	89.5	81.2	85.4	85.8	86.3	89.7	83.7
24	95.2	85.4	82.8	93.2	97.6	81.5	98.5	96.6	83.1	93.6	94.8	99.5	99.8	97.2

Table 6: in-vitro drug release studies at various time intervals.

Formulation	Factorial Amount (mg)		Rel_2 h (%)	Rel_{12} h (%)	Rel_{24} h (%)
	A	B			
F1	150	4	26.4	87.8	95.2
F2	200	5	23.7	73.7	85.4
F3	200	4	22.2	72.4	82.8
F4	150	3	26.8	84.4	93.2
F5	100	3	26.9	86.2	97.6
F6	200	3	21.5	63.4	86.1
F7	100	4	24.5	84.3	98.5
F8	150	5	22.1	82.5	93.6
F9	200	4	21.4	81.2	83.1
F10	150	3	27.8	85.4	93.6
F11	150	4	25.4	85.8	94.8
F12	100	5	25.5	86.3	96.5
F13	100	5	27.7	89.7	95.8
F14	100	3	24.7	83.7	97.2

The in-vitro drug release studies for the current investigations are mentioned in the table 5 which reflects an inverse proportionality for lecithin concentration to % drug release. The theory holds good for optimized concentrations of ethanol and when the same exceeds the limit, it enhances the membrane fluidization which in turn causes the vesicle leaky and enhances the drug release. The theory can be correlated to the results in which the drug release exhibits a direct proportionality with the ethanol concentration. But in view of the ongoing exploration, F6 should be considered as the optimized as it exhibits the least drug release. But on correlation to the other factors such as drug content and entrapment efficacy F6 is not much satisfactory and also there is a negligible difference in the in-vitro drug release between the F6 and F9 which enlightened us to consider F9 as the optimized. Further, in support to the ongoing discussion, the polynomial equations for the dependent variables such as Rel_2 h (%), Rel_{12} h (%), and Rel_{24} h (%) are developed and mentioned as follows:

Coded Equation for Rel_2 h (%) = 24.18 +0.7222A1 +1.26A2 + 0.48889B1 – 0.0778B2 – 0.6889A1B1 + 1.88 A2B1 – 0.3222A1B2 + 1.04A2B2

Coded Equation for Rel_{12} h (%) = 80.92 + 3.84A1 +4.31A2 – 3.42B1 + 3.51B2 + 2.36A1B1 + 3.59 A2B1 – 3.98A1B2 – 0.9444A2B2

Coded Equation for Rel_{24} h (%) = 92.13 + 5.27A1 +2.00A2 + 0.1667B1 + 0.1333B2 – 0.3667A1B1 - 0.7000 A2B1 + 0.9667A1B2 + 0.9333A2B2

In the above coded equations the positive sign indicates the enhancement of dependent variables and the negative sign indicates the decrease of dependent variables which justifies the impact of independent variables, lecithin and ethanol on the overall efficacy of the formulation.

Kinetic Studies:

Table 7: Kinetic parameters for various formulations.

Formulation Code	Kinetic Parameters			
	Zero Order Regression Coefficient	First Order Regression Coefficient	Higuchi Regression Coefficient	Korsemeyer peppas Regression Coefficient
F1	0.826	0.697	0.943	0.961
F2	0.813	0.675	0.952	0.966
F3	0.816	0.694	0.951	0.965
F4	0.810	0.671	0.952	0.967
F5	0.823	0.691	0.956	0.970
F6	0.879	0.725	0.980	0.983
F7	0.868	0.718	0.968	0.981
F8	0.831	0.710	0.943	0.970
F9	0.772	0.660	0.926	0.958
F10	0.805	0.673	0.950	0.967
F11	0.831	0.699	0.953	0.970
F12	0.863	0.721	0.967	0.980
F13	0.815	0.668	0.943	0.956
F14	0.862	0.720	0.967	0.979

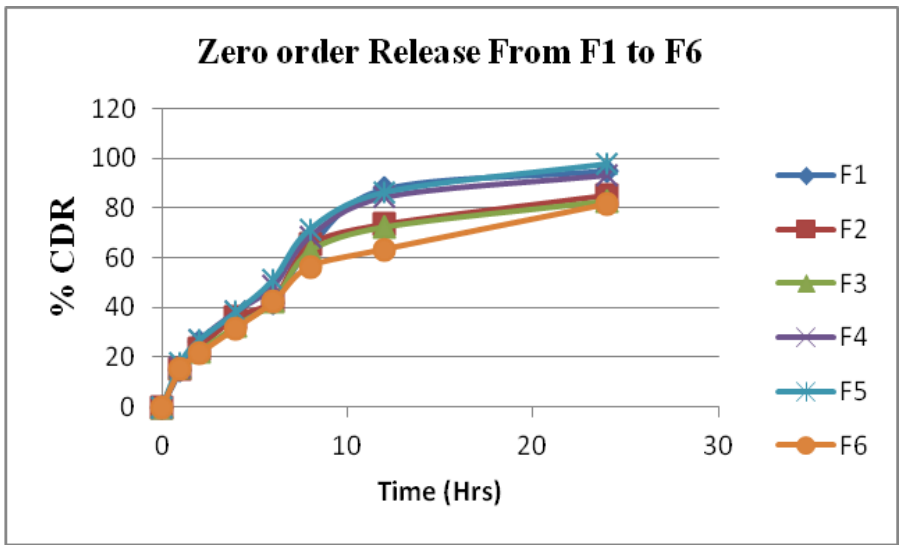


Figure1: Zero order release for formulations F1 to F6.

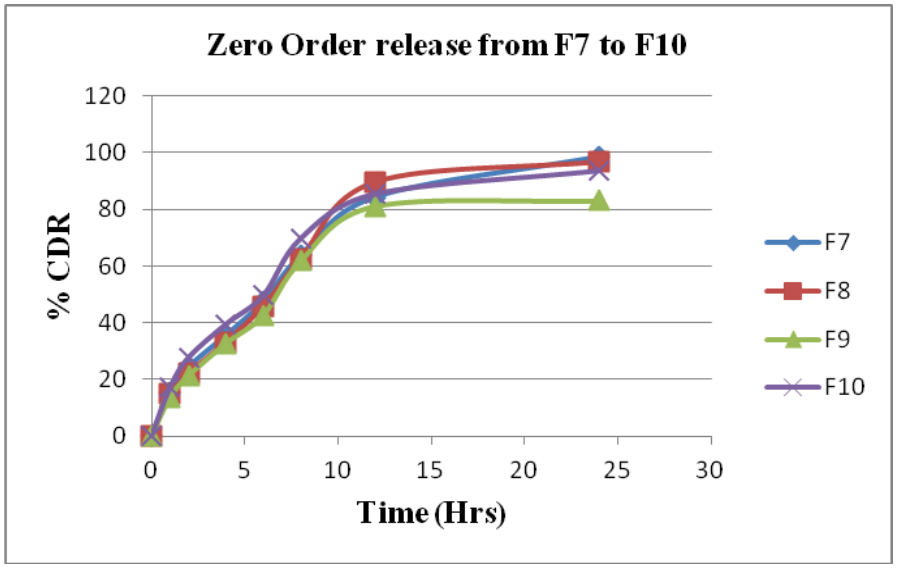


Figure2: Zero order release for formulations F7 to F10.

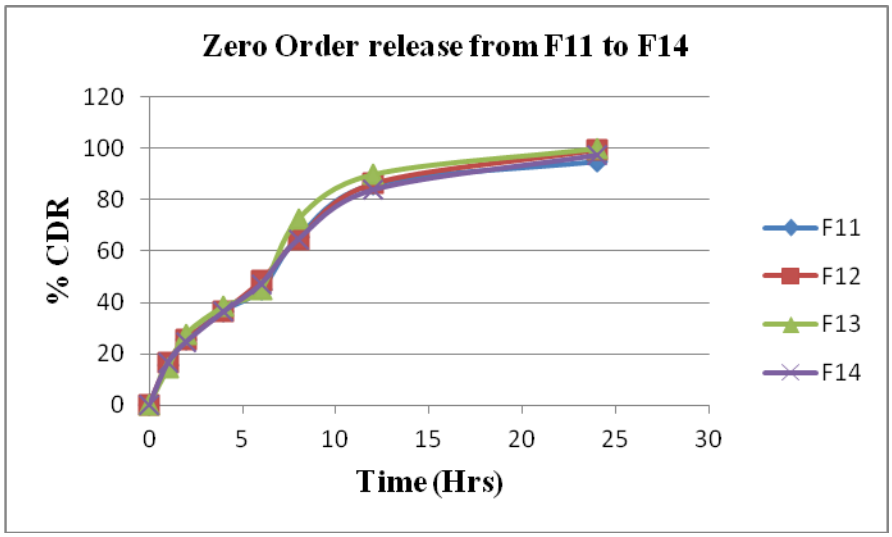


Figure 3: Zero order release for formulations F11 to F14.

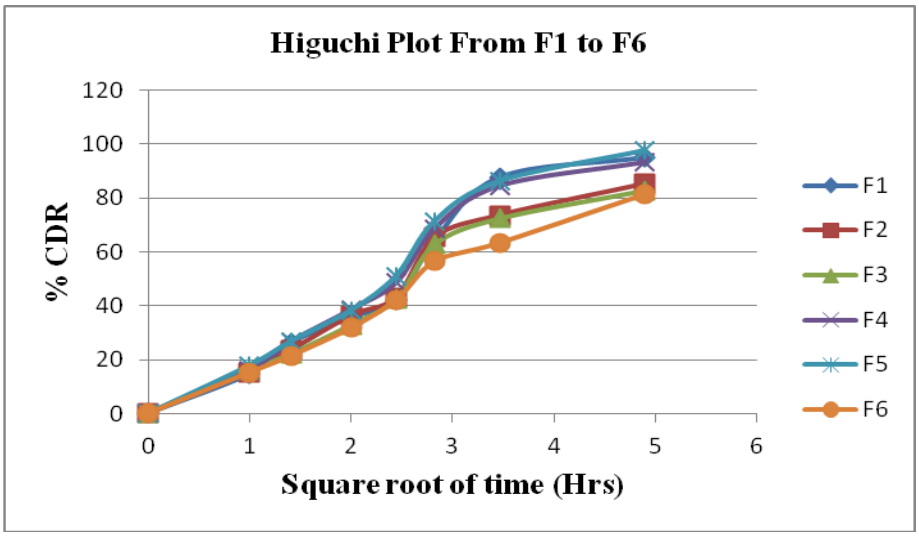


Figure 4: Higuchi plots for formulations F1 to F6.

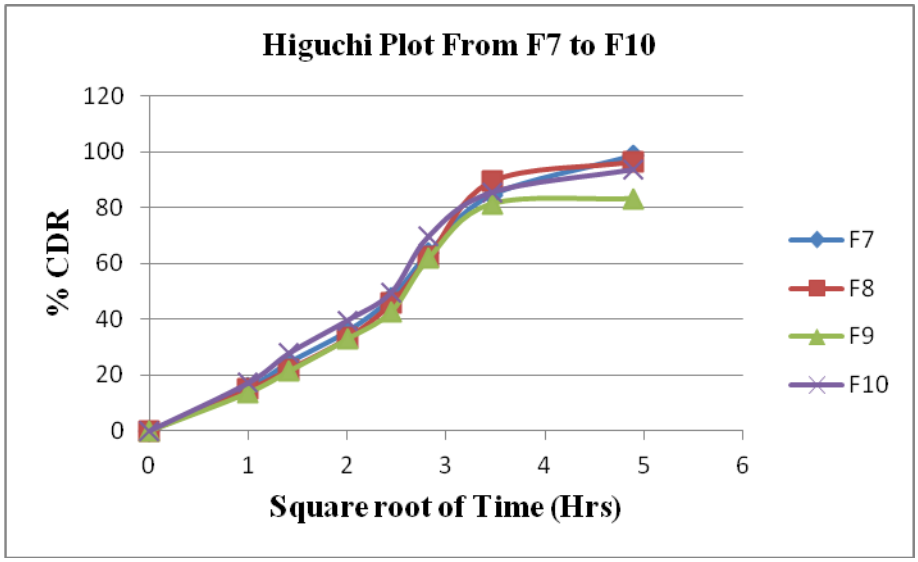


Figure5: Higuchi plots for formulations F7 to F10.

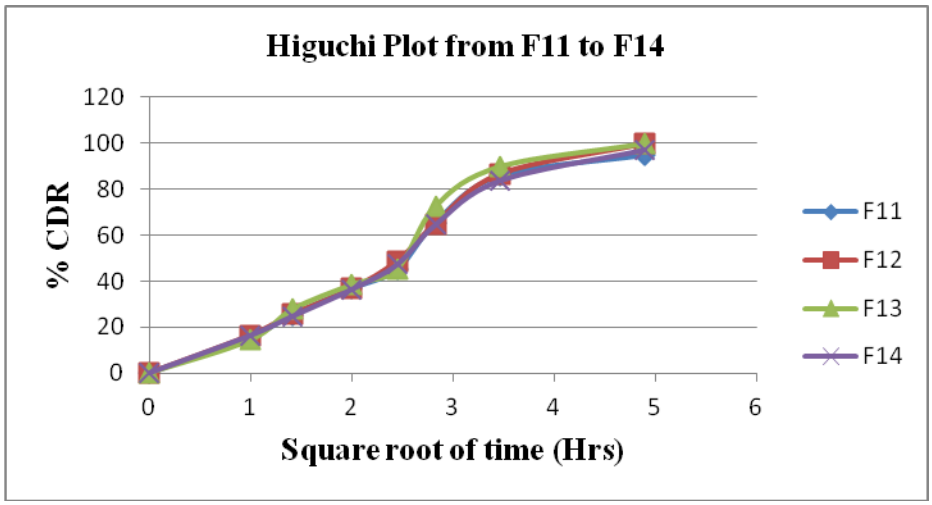


Figure 6: Higuchi plots for formulations F11 to F14.

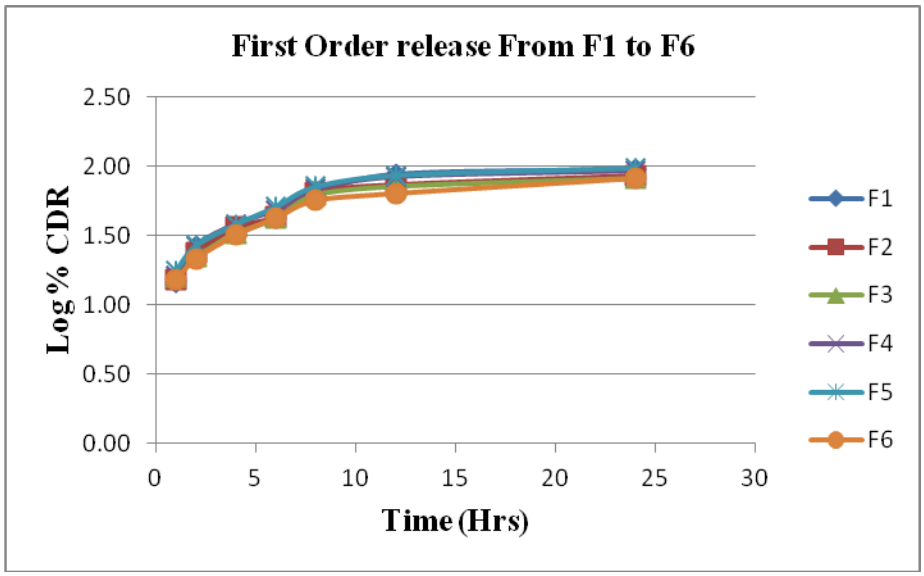


Figure 7: First order release for formulations F1 to F6.

Figure 8: First order release for formulations F7 to F10.

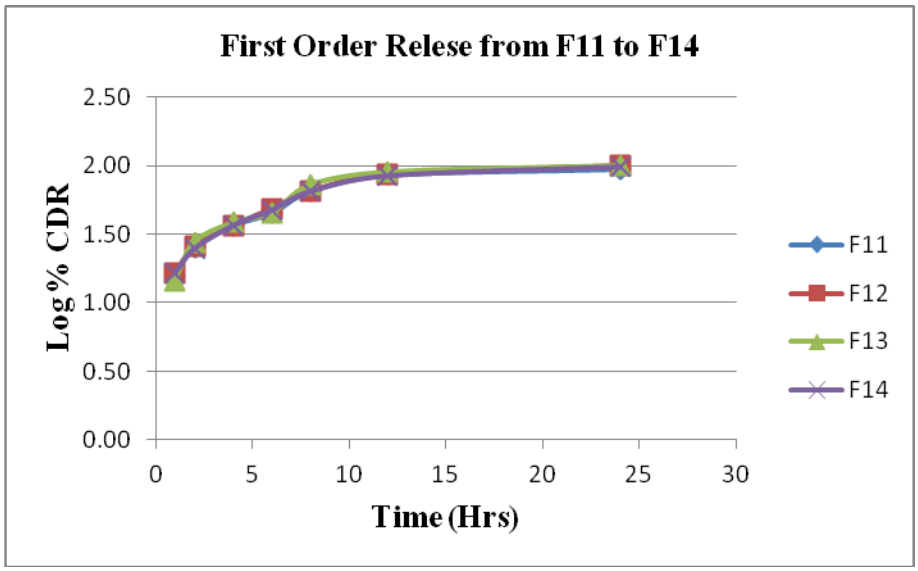


Figure 9: First order release for formulations F11 to F14.

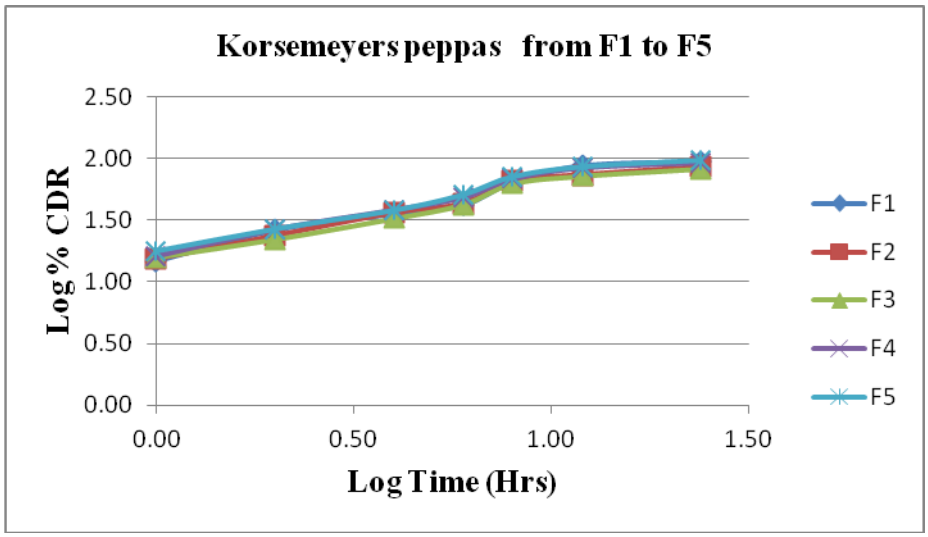


Figure 10: Korsemeyers's peppas model for formulations F1 to F5.

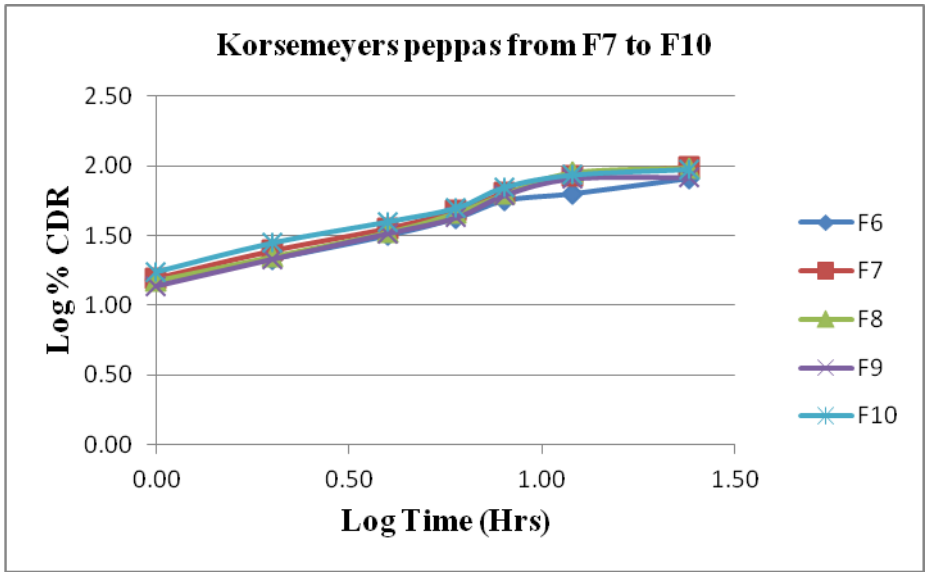


Figure 11: Korsemeyers's peppas model for formulations F7 to F10.

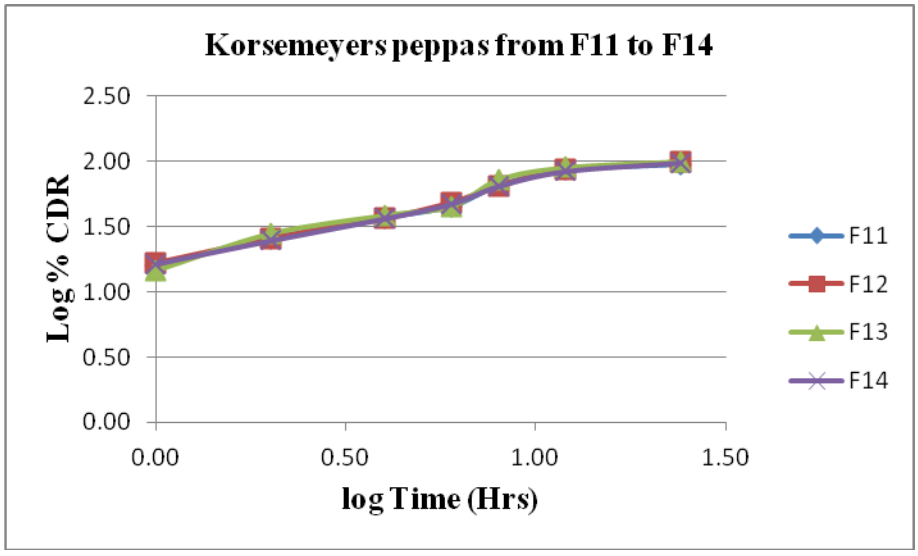


Figure 12: Korsemeyers's peppas model for formulations F11 to F14.

pH and Viscosity analysis:

The prepared formulations were transferred to gel by incorporating the specified concentrations of Ethosomes to carbopol D934 and maintaining its pH by drop by drop addition of Triethanolamine. The results depicted in table 8 reveal that the pH lie between 6.27 to 6.86 and are opted for application to the skin. Further, the viscosity determinations through brook field viscometer are mentioned in table 8 which enumerates that the prepared formulations does not cause any irritation to the skin and fits for transdermal administration.

Table 8 comparison of pH, viscosity, and vesicle size from F1-F14.

Formulation Code	pH	Viscosity (cps)	Vesicle size
F1	6.86	3200	11.42
F2	6.54	3487	13.65
F3	6.83	3549	15.67
F4	6.58	3684	9.31
F5	6.62	3675	6.37
F6	6.48	3851	12.37
F7	6.75	3756	8.64
F8	6.27	3682	10.28
F9	6.38	3986	15.82
F10	6.27	3482	9.28
F11	6.48	3678	11.39
F12	6.82	3982	7.35
F13	6.31	3754	7.54
F14	6.85	3914	6.48

ANOVA for Reduced Quartic model

Table 9: Entrapment Efficacy.

Model	262.78	8	32.85	3253.56	< 0.0001	significant
A-Lecithin	75.97	1	75.97	7524.95	< 0.0001	
B-Ethanol	1.01	1	1.01	99.82	0.0002	
AB	0.1487	1	0.1487	14.73	0.0121	
A^2	2.35	1	2.35	232.78	< 0.0001	
B^2	6.44	1	6.44	637.59	< 0.0001	
A^2B	0.0134	1	0.0134	1.33	0.3017	
AB^2	1.19	1	1.19	118.11	0.0001	
A^2B^2	0.0002	1	0.0002	0.0150	0.9073	
Pure Error	0.0505	5	0.0101			
Cor Total	262.83	13				

Table 9.1: Fit Statistics for Entrapment efficiency studies.

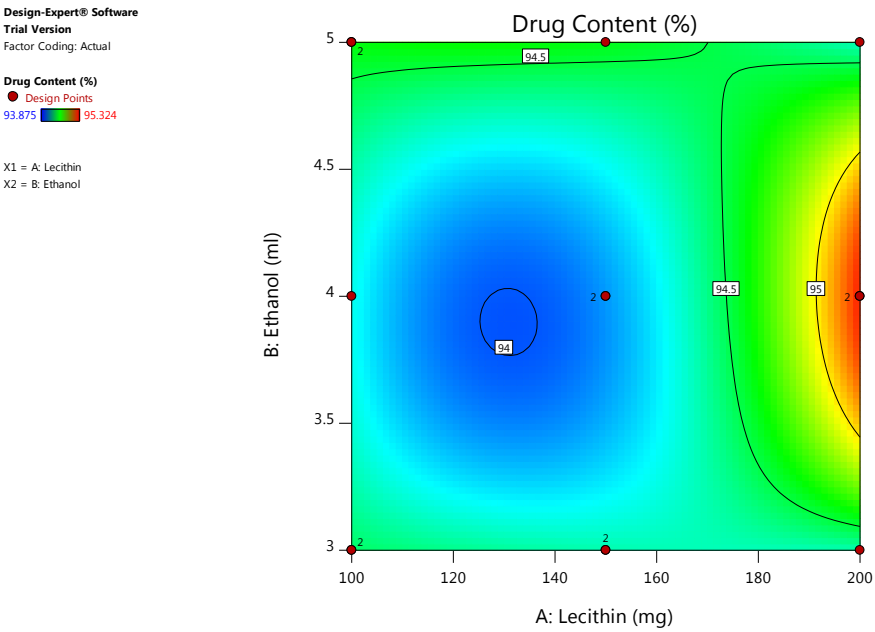
Std. Dev.	0.1005	**R^2**	0.9998
Mean	86.66	**Adjusted R^2**	0.9995
C.V. %	0.1159	**Predicted R^2**	NA[(1)]
		Adeq Precision	156.4643

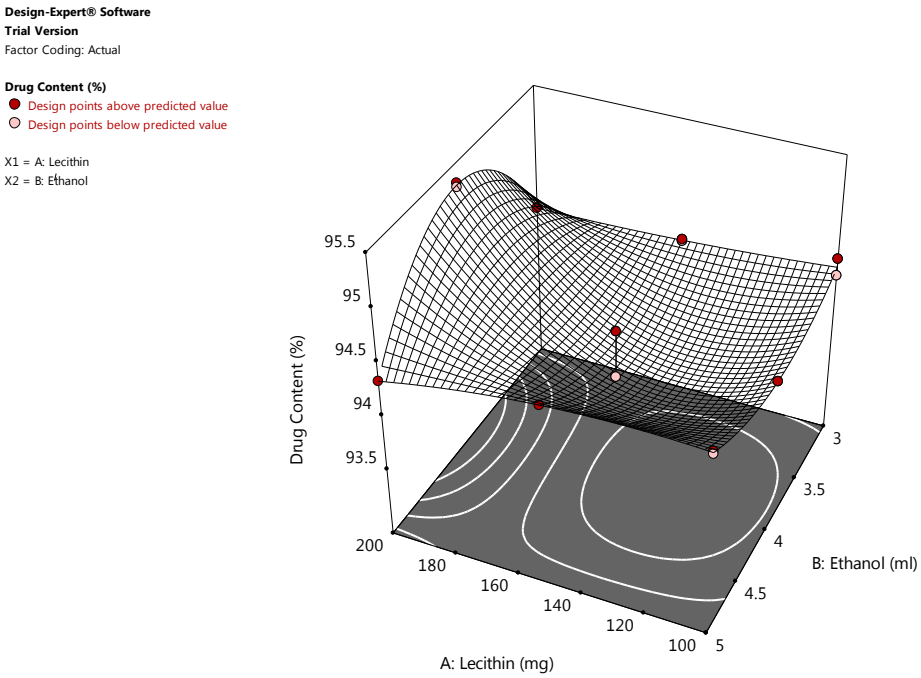
Table 10: Drug Content.

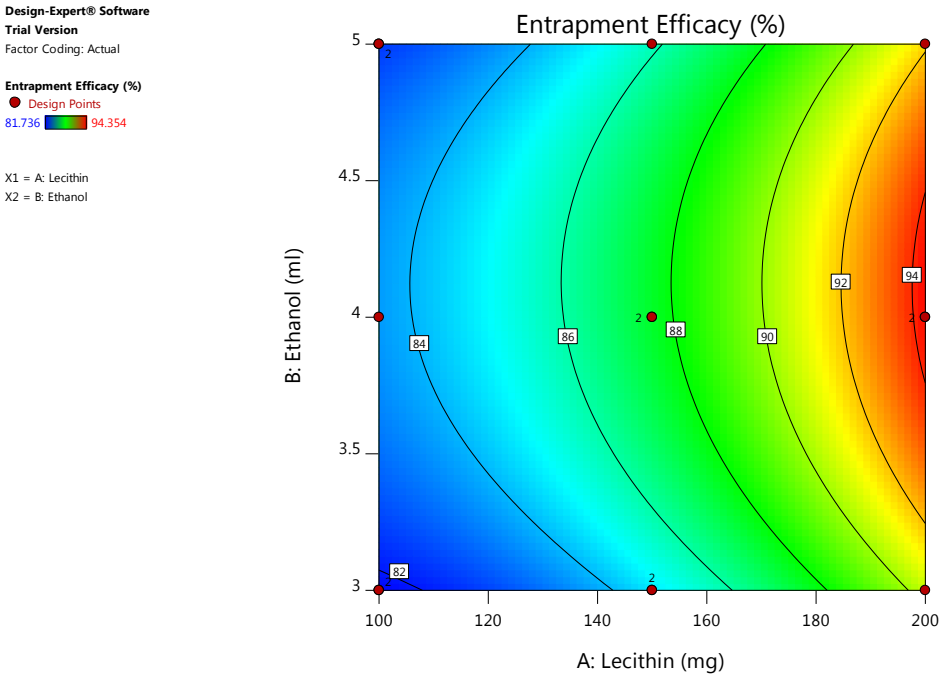
Model	1.79	8	0.2236	9.89	0.0109	significant
A-Lecithin	0.7357	1	0.7357	32.55	0.0023	
B-Ethanol	0.0269	1	0.0269	1.19	0.3248	
AB	0.0028	1	0.0028	0.1235	0.7396	
A^2	0.5312	1	0.5312	23.50	0.0047	
B^2	0.1506	1	0.1506	6.66	0.0494	
A^2B	0.0074	1	0.0074	0.3285	0.5914	
AB^2	0.6749	1	0.6749	29.86	0.0028	
A^2B^2	0.3530	1	0.3530	15.62	0.0108	
Pure Error	0.1130	5	0.0226			
Cor Total	1.90	13				

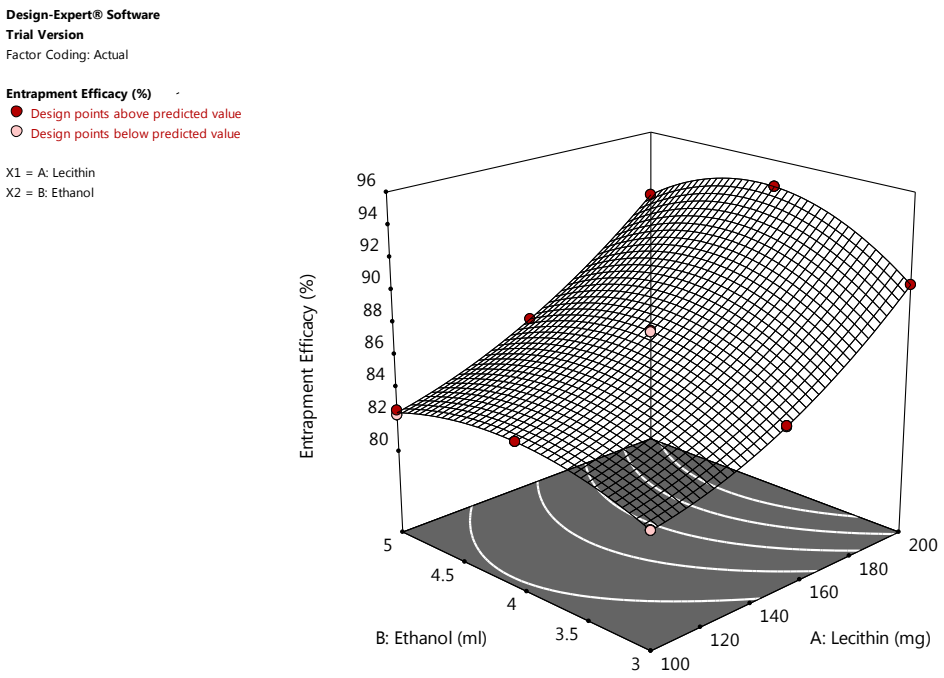
Fit Statistics.

Std. Dev.	0.1503	**R^2**	0.9406
Mean	94.51	**Adjusted R^2**	0.8455
C.V. %	0.1591	**Predicted R^2**	NA[1]
		Adeq Precision	10.0131









CONCLUSION

The current research is carried to trace out the optimized transdermal gel formulation of Paroxetine using cold method. The purpose of the investigation is to produce an optimized ethosomal formulation of Paroxetine for effective penetration of biological membranes and generate the required drug concentrations at the targeted site. The current research meets the required criteria through incorporation of an effective penetration enhancer, ethanol in varied concentrations and phospholipid lecithin which is hydrophobic in nature and effective in penetration and drug encapsulation. In connection to the above, the investigation preferred three square factorial design for producing an optimized formulation followed by in-vitro analysis to justify its efficacy and commercial productivity. Further, the obtained results upon subjecting to statistical techniques such as ANOVA revealed F3 as quite fit in fulfilling the predetermined quality attributes.

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Conflicts of interest:

The authors doesnot have any conflicts on interest.

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