Original Article

"Development And Optimization Of Microemulsion Based Transdermal Drug Delivery For Paroxetine Hydrochloride"

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Abstract

Background and Objectives: The aim of this work was to design and evaluate a microemulsion transdermal gel of Paroxetine Hydrochloride for the treatment of depression. Paroxetine is the most potent serotonin reuptake blocker antidepressant.

Methods: Phase-Titration method, D-Optimal design, Factorial design were used for the development of Paroxetine HCl microemulsions gel. Using pseudo-ternary phase diagram microemulsion area was selected and according to that ratio of % oil, % surfactant and % co-surfactant, microemulsion was optimized. Based on phase diagrams, formulations were prepared and evaluated for various parameters including compatibility test within the drug and excipient by FT-IR. The prepared microemulsion gel was subjected for various tests like size distribution study, zeta potential, DSC, % CDR, % transmittance, globule size, Cmax, Tmax, drug content, *in-vitro* diffusion and *ex-vivo* permeation studies.

Results: Microemulsion based gel of Paroxetine HCl formulations results revealed that all the physicochemical parameters were found to be desirable. The best formulation passed thermodynamic stability studies, robust to dilutions of different medium and showed drug release of approx. 65% in 8h. The optimized formulation having the combination of Flaxseed Oil (3ml), surfactant mixture consisted of Tween®80 & Propylene glycol in 1:3 ratio (17.06ml) and double distilled water (29.93ml) as an aqueous phase showed best physicochemical parameters with 60.82 % of *in-vitro* drug release at 8h i.e. close to predicted values obtained from d-optimal design. The optimized formulation showed no significant changes on physiochemical studies when subjected to accelerated stability studies according to ICH guidelines.

Interpretation and Conclusion: Based on the results it can be concluded that Microemulsion transdermal gel of Paroxetine Hydrochloride could be a potential candidate for the treatment of depression.

Keywords: Paroxetine Hydrochloride, Anti-depressant, Microemulsion, Flaxseed Oil, Formulations, and Transdermal gel.

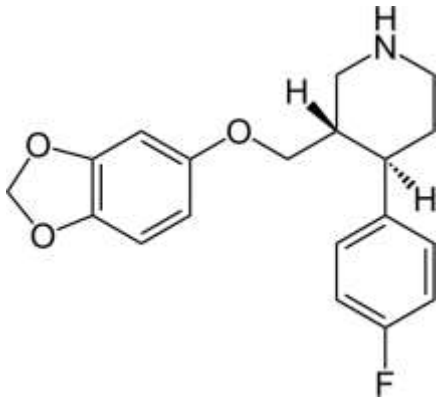
INTRODUCTION

Depression is may be the most common reason of emotional suffering over later life and considerably decreases quality of life among older adults. In current years, the literature of late-life depression has exploded. Many gaps between our appreciations of the consequence on late-life depression have been filled. Intriguing findings have emerged involving the aetiology of late-onset depression. The numerous studies documenting the evidence base for therapy has accelerated dramatically.[1] However; most antidepressants maintain numerous documented detrimental events, such as cardiometabolic consequences and weight gain, who are essential people health concerns. Current findings advocate the rational function of histamine and serotonin off-target appetite-promoting pathways into unfavorable weight-gain effects. Therefore, controlling for undesired measurement effects is an essential attention for the determination over antidepressants.[2] Paroxetine is a selective serotonin reuptake inhibitor i.e clinically used for the treatment on depression into human patients. Latest reports of the role on serotonin between modulating infection and the link into infection and depression, we sought in accordance with take a look at the effect of paroxetine directly of macrophage response to an inflammatory stimulus.[3] Transdermal drug transport has performed an integral exploit in accordance with scientific practice, however has but to wholly acquire its dynamic as an choice in conformity with oral delivery and hypodermic injections. First-generation transdermal delivery systems keep continued their constant extend in

scientific uses for delivery of small, lipophilic, low-dose drugs. Second-generation delivery systems the usage of chemical enhancers, noncavitational ultrasound and iontophoresis hold also resulted within clinical products; the ability concerning iontophoresis to control delivery charges in real period offers added functionality.[4]

Microemulsions have been considered as much as more tremendous topical vehicle than its conventional skin features like cream and gel. Being transparent and thermodynamically stabile system, microemulsions are made effortlessly with relative amenities on manufacture. Such system has greater reach up dynamic demonstrating their manufactured feasibility as well. These nano-structured vehicle exhibited better solubilization of drug, higher skin permeation concerning medicine of assessment to traditional formulations now utilized on skin. Enhanced medicine solubilization, expanded flux throughout skin, decrease of diffusion co-efficients are most important attributes concerning microemulsion system remaining according to inner phase existed of nano-size droplet, ultralow interfacial tension with superior surface free energy.[5] The current study is to develop ideal Flaxseed oil microemulsion based Paroxetine Hydrochloride drug delivery by transdermal drug delivery system. Statistical design and optimization of the formulation, evaluation of various physicochemical properties, penetration efficiency and drug release have been developed to enhance transdermal drug delivery for therapeutic purposes.

1.1 Drug Profile of Paroxetine Hydrochloride[6]



Paroxetine Hydrochloride | C19H21ClFNO3

IUPAC Name: (3S, 4R)-3-[(2H-1, 3-benzodioxol-5-yloxy) methyl]-4-(4-

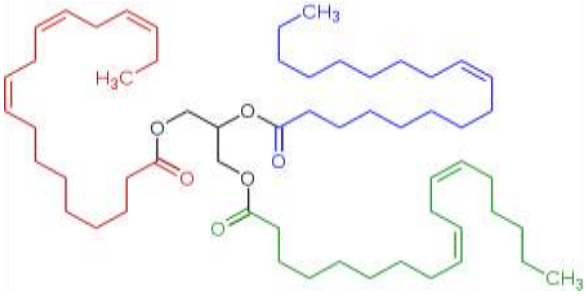
Fluorophenyl) piperidine

1.2 EXCIPIENT PROFILE[7-9]

Flaxseed Oil, Linseed Oil (Common Name)

Formula and Molecular weight: C26H38O12 and 542.6g/mol

Str. Formula:



Properties:

Acidity/alkalinity: pH = 6-8 (for viscous of flaxseed mucilage), Boiling point: >316ºC, Melting point: -24.0ºC, Refractive index: n20/D 1.4795(lit.), Flash point : >230ºF, Solubility: As a liquid, it is soluble in ether, chloroform, carbon disulfide, ligroin and turpentine. When dry, it is insoluble in most solvents, Saponification number: 190-193. Density: 0.93 g/mL at 25ºC.

MATERIALS AND METHOD

2.1 Materials and Reagent

Drug: Paroxetine HCL (Procured from: Yarrow Chemical Products, Mumbai, Maharashtra, India), **Oil:** Flaxseed Oil (Sigma Aldrich), **Surfactant and co-surfactant,** (Yarrow Chemical Products) Tween 80 (Formula: $C_{64}H_{124}O_{26,}$ Molar Mass: 1310 g/mol, Density, 1.102 g/mL) and Propylene glycol (Formula: C3H8O. Molecular weight: 76.0944, Density: 1.04 g/cm^3, Molar mass: 76.09 g/mol).

2.2 Equipment

Electronic analytical balance (Shimadzu AUX -224), Magnetic stirrer balance (Remi instruments private limited, Mumbai), Vortex mixture (Remi instruments private limited, Mumbai), Centrifuge (Remi instruments private limited ,Mumbai), Shaker (DBN instruments, Bangalore), UV visible spectrophotometer (UV -1700, shimadzu corporation, Japan), Droplet size analyser (Brookhaven zetapals), Zeta potential analyser (Brookhaven zetapals), Brookfield viscometer (Brookfield LVD III+CONE), Deep freezer (Blue star), FTIR (Brucker), pH meter (Consolidated electrical industries, Bangalore), Hot air oven (Remi instruments private limited, Mumbai), Refrigerator (Videocon), Stability chamber (Thermolab), Electronic microscope (Labomedi VU 3000).

2.3 Authentication of Drug

2.3.1 Identification of Drug by Melting Point analysis: The melting point of pure Paroxetine was determined by open capillary method.[10]

2.3.2 Solubility Determination: Solubility of Paroxetine Hydrochloride was determined in methanol, ethanol, acetonitrile, PBS pH 7.4 etc. Solubility studies were performed by taking some amount of Paroxetine Hydrochloride in different test tubes containing the solvent.[11]

2.3.3 Identification and Estimation of Drug by UV-Vis Spectrophotometry: Preparation of PBS of pH 7.4, 2.38g of Na_2HPO_4, 0.19 g of K_2PO_4 and 8.0g of Nacl were dissolved in q.s. DW to produce 1000mL and adjust the pH.

2.3.4 Determination of λ max: A primary stock solution of concentration 10 µg/mL was prepared by accurately weighing 100 mg of drug (paroxetine) and dissolving it in 100 mL of ethanol. Further from the above solution 1ml was made up the volume of ethanol with 100ml. The prepared stock solution was scanned over the range of 200-400 nm against PBS (pH 7.4) as blank using Shimadzu double beam UV-visible spectrophotometer 1900-I .

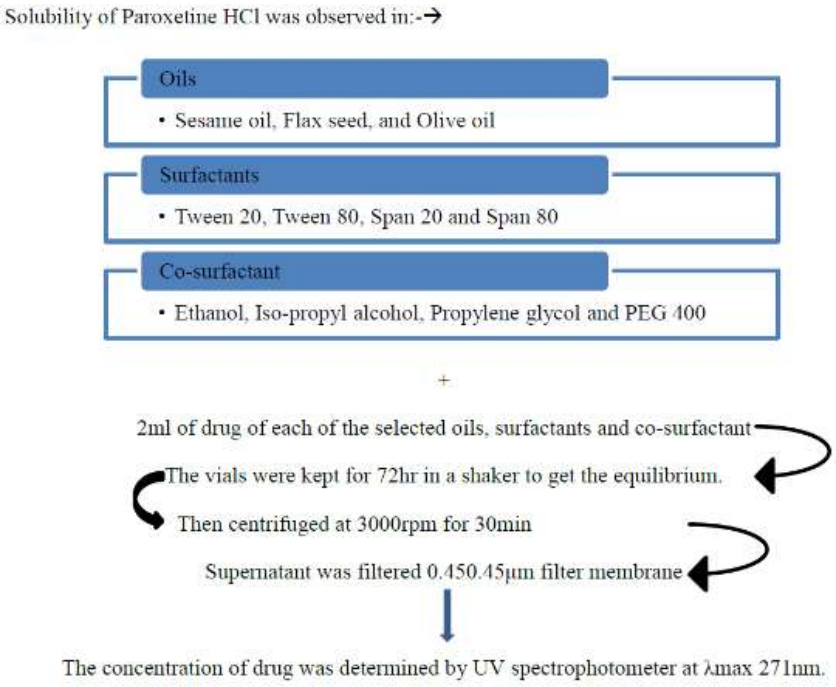
2.3.5 Standard stock solutions of Paroxetine HCl in Ethanol: Accurately weighed 100mg of standard Paroxetine HCl was dissolved in 100mL of Ethanol.

2.3.6 Preparation of Working Standard: From standard stock solution, different concentrations of aliquots varying between 2-20µg/mL were prepared. The standard solutions were made by proper dilution of the stock solution with Ethanol in a concentration range of 2µg/mL, 4µg/mL, 6µg/mL, 8µg/mL and 10µg/mL. The absorbance was measured at 271 nm using UV Visible spectrophotometer by plotting absorbance vs. concentration in µg/mL, the standard.

2.3.7 Identification of Drug (Paroxetine HCL) by FTIR Spectroscopy: FT-IR Spectrum obtained for the pure drug and its corresponding interpretation for identification of functional groups and bonds.

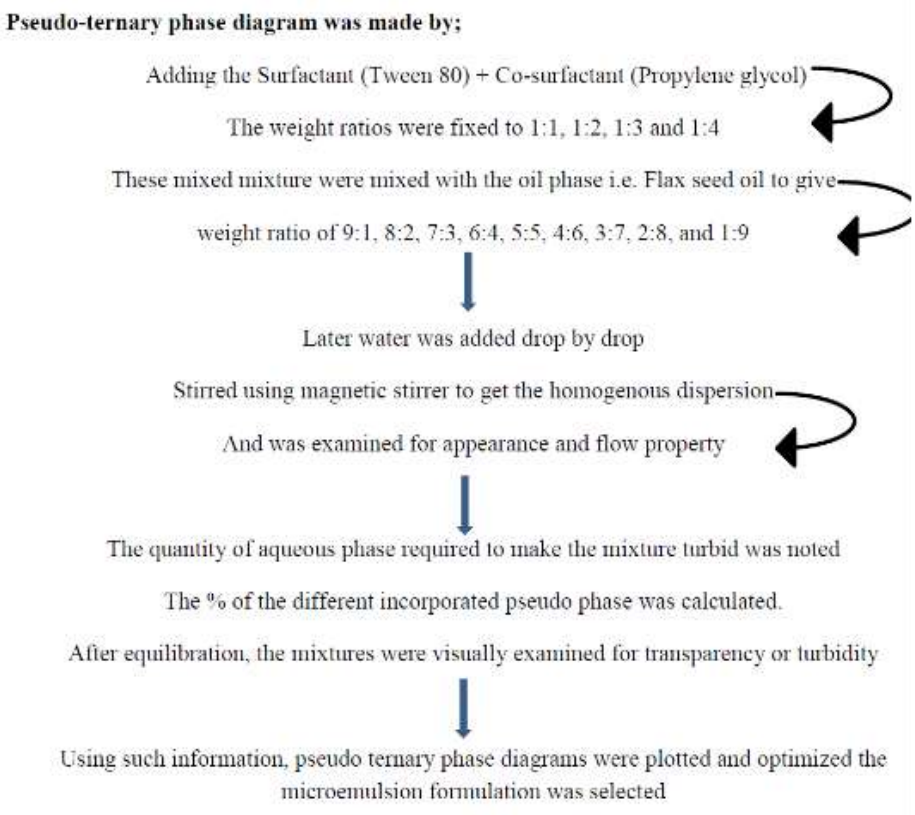
2.4 Pre-Formulation Compatibility Studies

2.4.1 Selection and Screening of Oils, Surfactants and Co-surfactant for Microemulsion components:[12]



2.5 Construction of pseudo-ternary phase diagrams

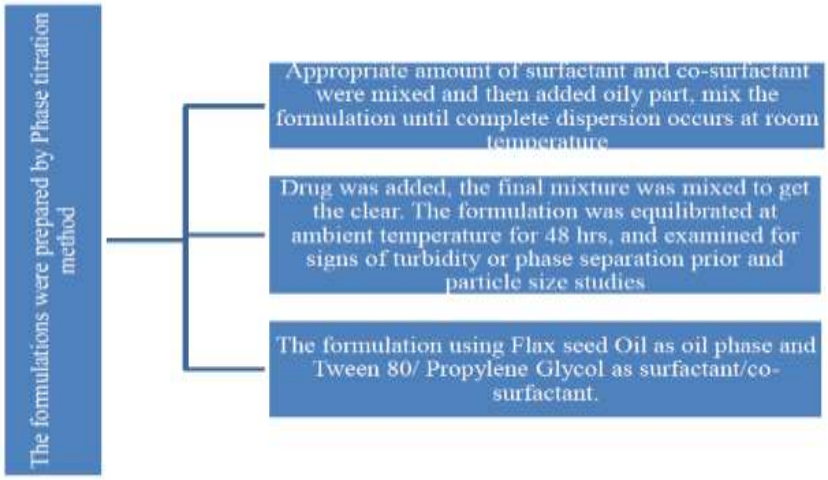
Pseudo Ternary Phase Diagram was constructed by using Oil, Surfactant and water ratio with various combinations from the literature and solubility study to perform the Preformulation studies to formulate Paroxetine HCl microemulsions. The microemulsion existence region was employed by Titration method and constructed pseudo-ternary phase diagrams.



2.5.1 Drug-Excipient Compatibility studies by FTIR:

FTIR Spectrophotometer with ATR (Bruker Alpha series) was used to carry out the compatibility study between pure drug and the excipient used to make microemulsion in house (KCP, Analytical Lab, Bangalore).

2.6 Formulation of Paroxetine Microemulsion systems:



2.7 Design of Experimentation

2.7.1 D-Optimal design for Paroxetine microemulsion

Paroxetine loaded Flaxeed oil microemulsions were prepared using D-Optimal Design. Design-Expert® software (Trial Version 13, Stat-Ease Inc., MN, USA) was used for analyzing the experimental design in order to choose the optimal formula. In current study, formulation variables: oil phase X1, Smix X2 and aqueous phase X3 were chosen as independent variables, while globule size (Y1), Transmittance (Y2), Drug Permeability at 6 Hrs (Y3) and Drug Permeability at 12 Hrs (Y4) were selected as dependent variables with Flaxseed Oil 3-5 %, S-Mix 15-50 % and Water 45.74 - 81.52 % as variable concentrations. The optimal Paroxetine microemulsions formulation has been chosen by applying the desirability function. The independent variables and the responses were characterized and summarized. The optimization method was intended to obtain a formula with Range specification of Globule size, Transmittance, Drug Permeability at 6 Hrs and Drug Permeability at 12 Hrs. The solution with desirability value near to one was selected.

2.7.2 Factorial Design for Ionotophoretic effect of Paroxetine Microemulsion

The optimised Paroxetine microemulsions formulations were fabricated subjected to perform a 2^2 factorial design. Design-Expert® software (Trial Version 13, Stat-Ease Inc., MN, USA) was used for analyzing the effect of ionotophoresis on Paroxetine microemulsions experimental design;

Current Density (X1), Time of Current Application (X2) were chosen as independent variables, while Cmax ie (Maximum Drug Release) (Y1), Tmax (Time at Maximum Drug Release) (Y2), Drug Content (Y3) were selected as dependent variables. The optimised formulation prediction was chosen by selecting range of specific values as desirability function.

2.8 Method of preparation of Paroxetine loaded microemulsion based transdermal gel

Preparation of gel: Drug Phase: Paroxetine HCL Microemulsion, **Aqueous Phase**: Carbopol 934 + water hear & stirring in a magnetic stirrer then add Triethanolamine 2ml to 4 ml as drops. **Formulation of gel base:** The microemulsion formulation of paroxetine was incorporated into 2% of carbopol 934 to get a gel of microemulsion. Weighed quantity of the carbopol 934 was soaked in distilled water for 2hrs. The best and stable microemulsion was incorporated and mixed thoroughly and the pH was adjusted to neutral with triethanolamine.

2.9 Evaluation of Paroxetine HCL Microemulsion and Microemulsion based Transdermal gel[14-19]

2.9.1 Thermodynamic stability:

a) Heating cooling cycle (Storage temperature between 4˚C and 45˚C for 48 hrs. was studied). b) Centrifugation (At 3500 rpm for 30min, sample did not show any separations have been taken for further test). c) Freeze thaw cycle (Between -21˚C and +25˚C for 48h).

Those formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility test for assessing the efficiency of self-emulsification.

2.9.2 Quantitative Test:

a) % Transmittance Test
% Transmittance of samples has been measured at 650nm through UV Spectrophotometer.

b) Determination of Drug Content
The 10µg/ml Paroxetine HCl microemulsion formulation was dissolved in ethanol and measured at 271 nm through UV spectroscopic method.

2.9.3 Observation of Microemulsion by Optical Microscope

Optical microscope of Globule size of Microemulsion of Flaxseed Oil
Mean globule size was determined through Malvern Zeta sizer.

The sample of 1.0gm was dissolved in solvent to get homogenous dispersion and injected to photocell of zeta sizer.

2.9.4 Measurement of Droplet Size
The measurement of droplet size was done by Malvern zeta sizer. The polydispersity value described the homogeneity of the droplet size; if the values are low this indicates uniformity of droplet size.

2.9.5 Particle size distribution Analysis
The particle size distribution analysis was used by dynamic light scattering (Malvern ZS instrument). It was carried out by dilutions in MQ H_2O at 25.1°C for 70sec.

2.9.6 Determination of Zeta potential
It is measured by Malvern zeta size.

The rational of the zeta potential is to control the charge interaction which can be related to the stability of colloidal dispersions.

2.9.7 Thermal Analysis

2.9.7.1 Differential scanning Colorimetry (DSC)
The drug status in the physical mixture was investigated by using a DSC Shimmadzu, which covered the heating range of 0°C to 300°C and 10°C/min of cooling rates. The melting point and glass transition temp. were recorded from the endothermic peak of DSC curve.

2.9.8 *In-Vitro* Diffusion Study
The drug release rates of different formulation were determined by using Franz diffusion cells with cellulose membrane (it has to be hydrates in DW at 25ºC for 24 hrs.). The membrane was clamped between the donor and receptor compartments of the diffusion cells, which was prefilled with PBS, pH 6.8, 130 ml. The receptor fluid was constantly stirred magnetically at 300 rpm throughout the experiment. The 5gm of formulation was weighed and kept in donor compartment and then 5ml sample was taken out from the receptor cells at intervals time of 1, 2, 3, 4, 5, 6, 7, and 8 h for analysis and quickly replaced with an equal volume of fresh receptor medium. Samples were analyzed by UV visible spectrophotometer at 271nm.

The results were plotted as CRD % vs. time.[20]

2.9.9 *Ex-Vivo* transdermal Permeation study

Fig. 1: Franz diffusion cell with porcine skin

It is done by using Franz diffusion (Effective area of 7.1 cm2) cell. The porcine skin is placed between the donor and receptor compartment with the stratum corneum facing donor compartment. The receptor chamber was pre-filled with PBS and maintained the temperature at 37±10ºC and stirred magnetically at 50 rpm. Samples are taken out at predetermined time intervals and analysed by using UV spectrophotometer.[20]

(Note: Fresh PBS was replaced in the receptor chamber after each sampling)

2.10 Accelerated Stability Studies

It was carried out on optimized formulation according to ICH guidelines to ensure their shelf life. The optimized formulation was kept in amber coloured glass with air tight closures and stored at 40°C and 75% RH for 3 months and analysed at 1 month intervals.[21]

STATISTICAL ANALYSIS

The data was analysed by: By using Design Expert Software, D-Optimal design for development of microemulsion, factorial design for effect of Ionotophoresis and one way ANOVA followed by 3D Graph.

RESULTS

4.1 Identification of Drug (Paroxetine) by Melting Point analysis:

The melting point of pure Drug was found to be 128ºC.

4.2 The solubility

The solubility of the selected drug was determined with different solvents and found to be soluble in Ethanol, Methanol, and Acetonitrile and partially soluble in PBS pH 7.4, Insoluble in Water and PBS pH 6.8.

4.3 Determination of λmax

λmax of Paroxetine Hydrochloride was found to be 271 nm.

4.3.1 Standard Calibration Curve for Paroxetine HCl with PBS, pH 7.4.

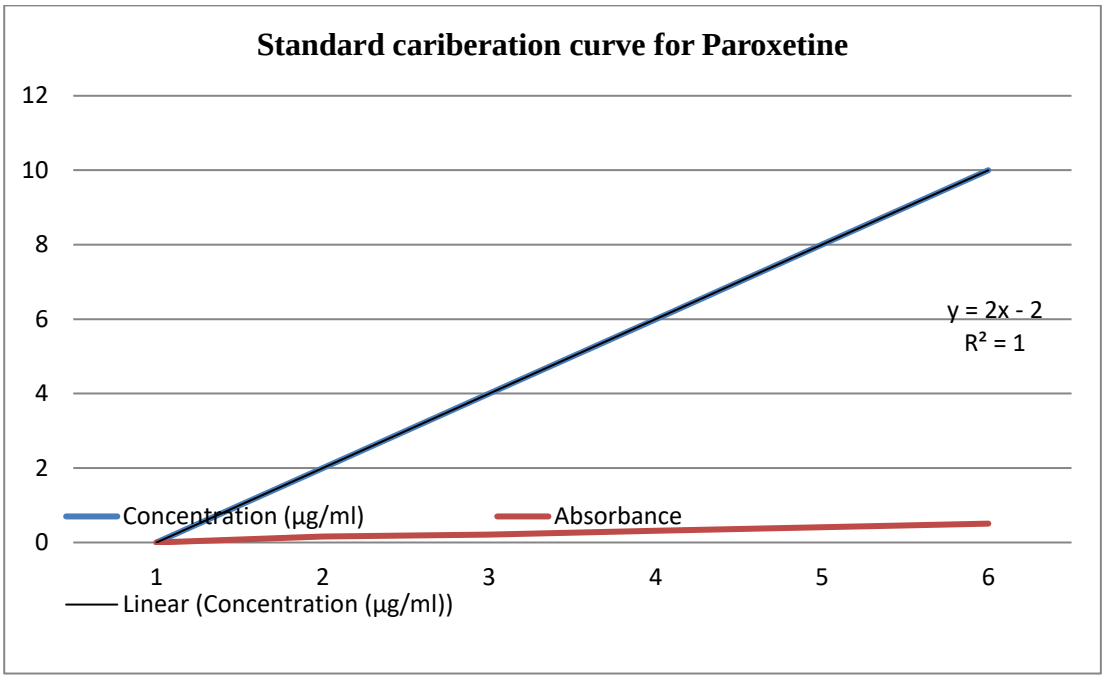


Fig.02: Std. Calibration curve Paroxetine HCl

4.3.2 Standard Calibration Curve for Paroxetine HCl in Ethanol

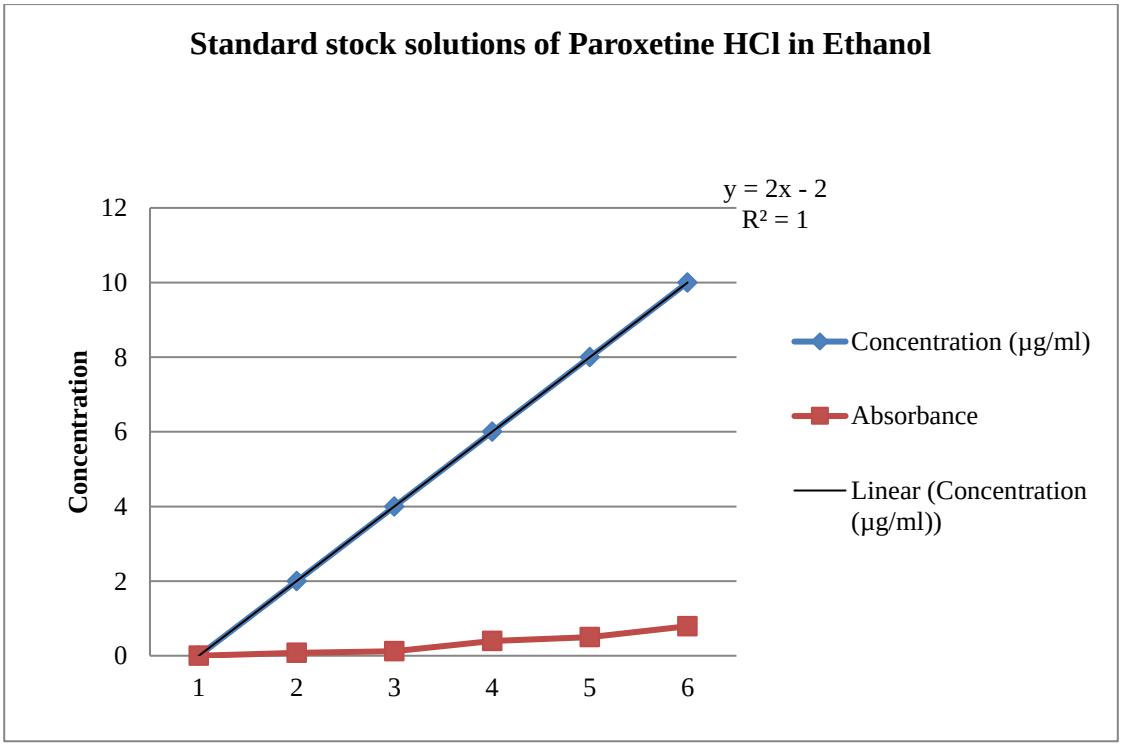


Fig. 03: Std. Stock Soln. of Paroxetine HCl in Ethanol

4.4 Identification of Drug (Paroxetine HCl) by FTIR Spectroscopy

The FTIR spectrum of Paroxetine Hcl is presented in Fig. 04 and its corresponding interpretation for identification of functional groups and bonds is given in Table 01.

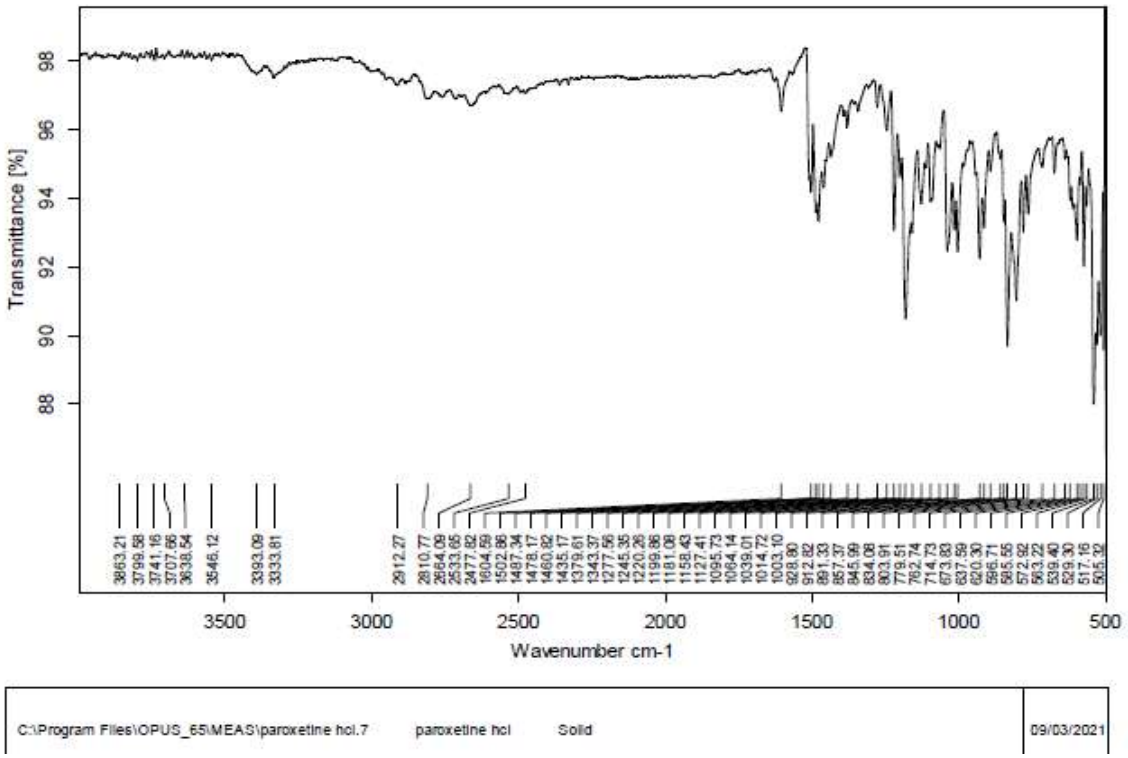


Fig. 04: FTIR Spectrum of Paroxetine HCl

4.5 Pre-Formulation Compatibility Studies

4.5.1 Selection and Screening of Oils, Surfactants and Co-surfactant for Microemulsion components;

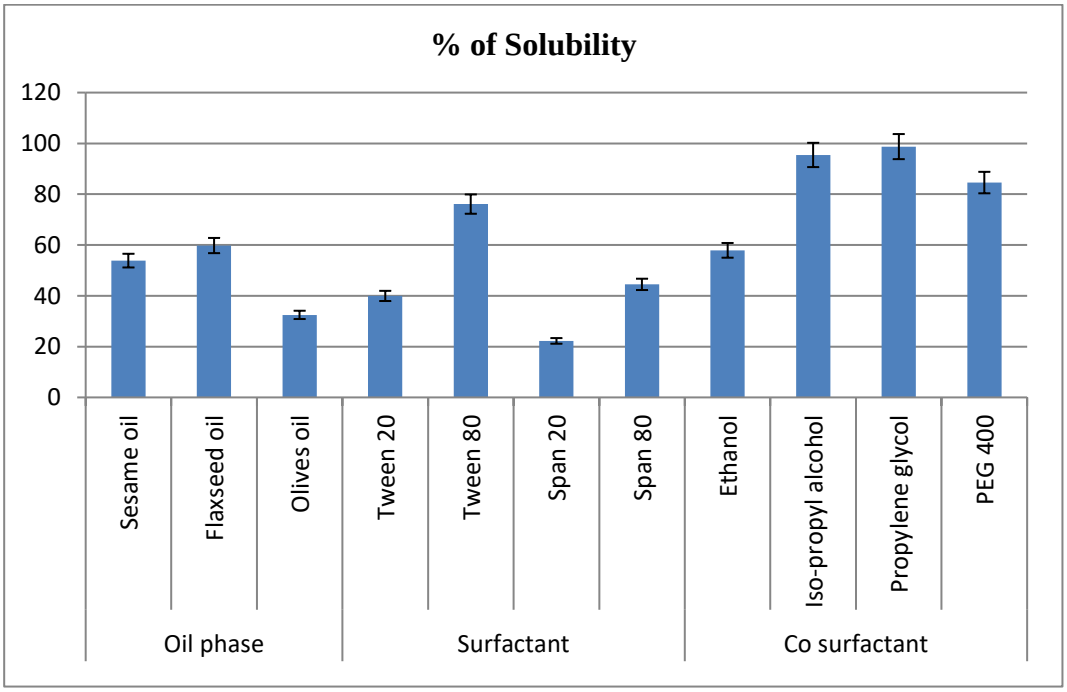


Fig. 05: Schematic diagram of drug solubility in different Oil, Surfactant, and Co-Surfactant

The Maximum solubility of drug candidate in Oil phase, Surfactant and co-Surfactant was found to be in Flaxseed Oil (59.79%), Tween 80 (76.11%), and Propylene glycol (98.74%) respectively, which has been taken for the formulation of microemulsion.

4.5.2 Construction of pseudo-ternary phase diagrams

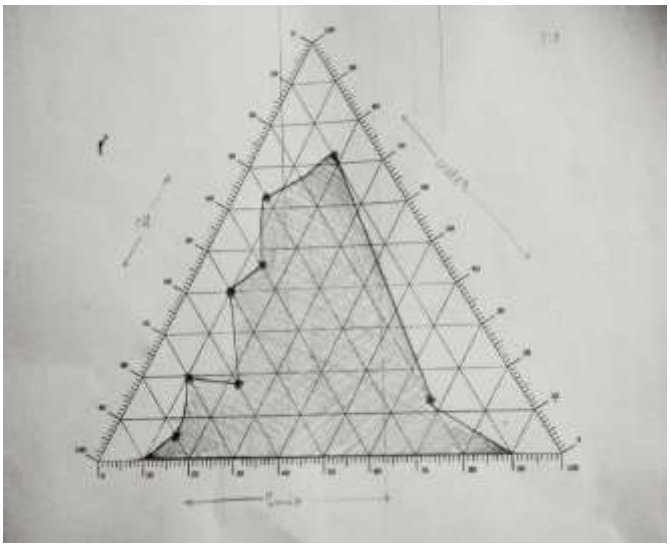


Fig. 06: Pseudo ternary phase diagrams for Microemulsion: Oil phase consisted of Flaxseed Oil (3ml), surfactant mixture consisted of Tween®80 & Propylene glycol in 1:3 ratio (17.06ml) and double distilled water (29.93ml) as an aqueous phase. Dark shaded region indicated Microemulsion region.

4.6 Drug-Excipient Compatibility studies by FTIR:

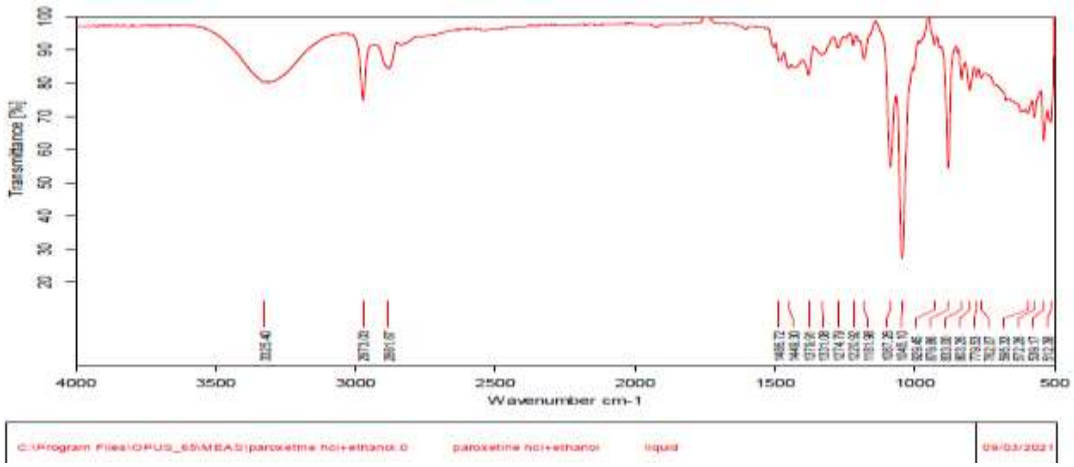


Fig. 07: FT-IR of Paroxetine with Ethanol

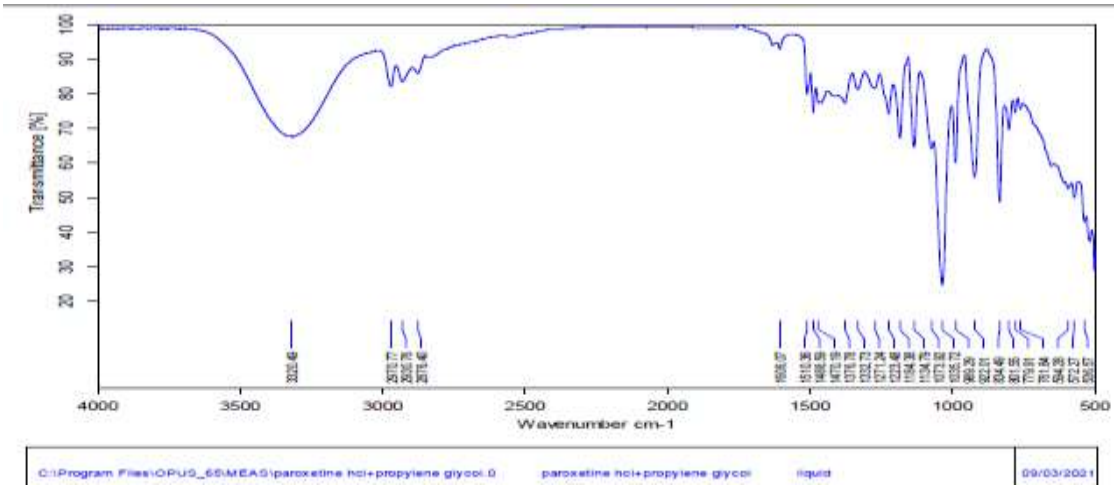


Fig. 08: FT-IR of Paroxetine with Propylene Glycol

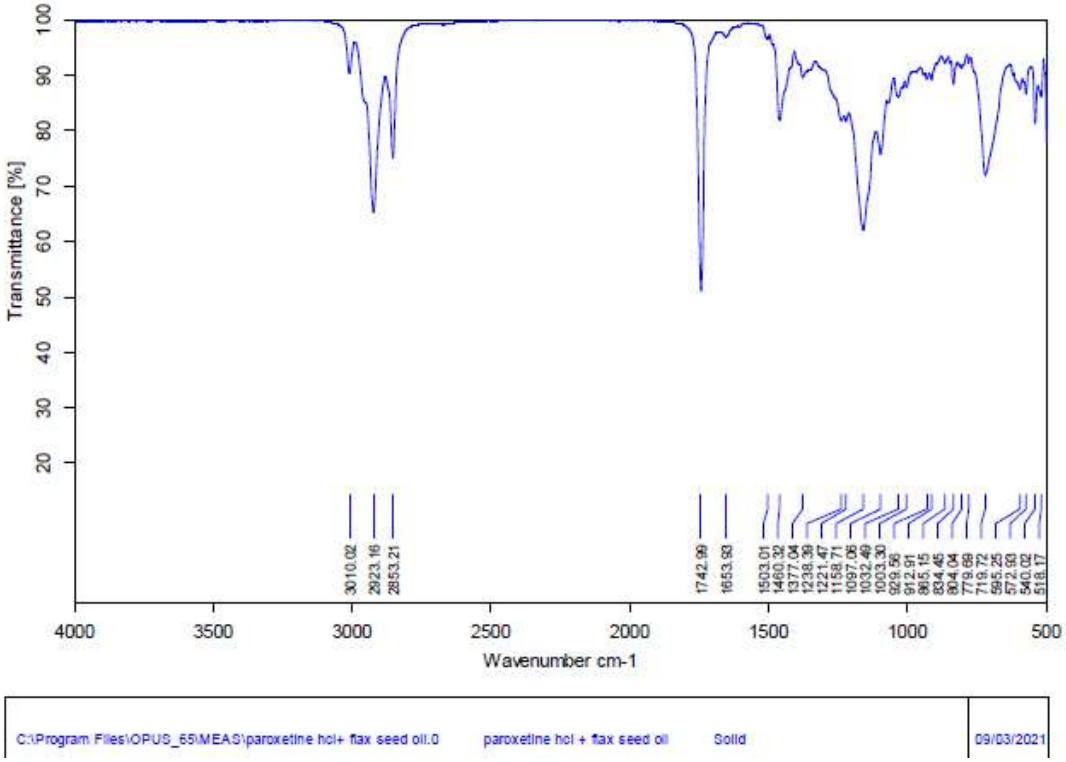


Fig. 09: FT-IR of Paroxetine with Flaxseed Oil

Table 01: Interpretation of FT-IR Spectra of Paroxetine

Functional group	N-H Stretching	O-H Stretching	N-H Bending	C-N Stretching
Range wave number (cm-1)	3400-3250	3300-2500	1650-1580	1250-1020
Observed value in Paroxetine HCl	3333.81	2533.65	1604.59	1095.73
Paroxetine HCl+ Ethanol	3325.40	-	-	1087.26
Paroxetine HCl + Propylene Glycol	3320.49	2876.40	1606.07	1035.72
Paroxetine HCl + Flaxseed Oil	-	2853.21	-	1032.49

By the interpretation of FT-IR, it was clear that there were no changes in the main peaks of drug and hence, confirming there was no physical interaction between them.

Table 02: Thermodynamically Stability study data for Formulation:

Sl. No.	Heating cooling cycle	Centrifugation test	Freeze thaw cycle	Inference
1	√	√	√	√
2	√	√	√	√
3	√	√	√	√
4	√	√	√	√
5	√	√	√	√
6	√	√	√	√
8	√	√	√	√
9	√	√	√	√

4.7 Formulation of Factorial expert design of Flaxseed Oil

Table 03: Build Information

File Version	13.0.5.0		
Study Type	Mixture	**Subtype**	Randomized
Design Type	D-optimal Coordinate Exchange	**Runs**	9.00
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	48.00		

Table 04: Characterization of Microemulsion by Factorial Model

	Component 1	Component 2	Component 3	Response 1	Response 2	Response 3	Response 4
Run	A: Flaxseed Oil	B:S-Mix	C:Water	R1-Globule Size	R2-Transmittance	R3 - Drug Permeation in 6 Hrs	R4 - - Drug Permeation in 12 Hrs
	ml	ml	ml	nm	%	%	%
1	5	22	23	193.21	95.91	40.64	68.19
2	3	22	25	185.14	97.85	38.81	64.73
3	5	15	30	195.26	95.14	38.3	69.22
4	4.07929	22	23.9207	189.05	96.75	38.54	68.06
5	4	18.5	27.5	185.26	96.15	37.43	67.04
6	5	18.6767	26.3233	197.35	95.48	39.05	69.25
7	5	15	30	197.66	95.19	38.24	68.66
8	3	17.0644	29.9356	180.56	97.49	37.37	64.74
9	3	15	32	185.83	97.12	36.43	67.04

Table 05: Summary of ANOVA for measured Response:

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio	F-value	p-value
R1	R1-Globule Size	nm	9.00	180.56	197.66	189.92	6.17	1.09	28.30	0.0345
R2	R2-Transmittance	%	9.00	95.14	97.85	96.34	1.01	1.03	53.22	0.0186
R3	R3 - Drug Permeation in 6 Hrs	%	9.00	36.43	40.64	38.31	1.20	1.12	486.45	0.0021
R4	R4 - - Drug Permeation in 12 Hrs	%	9.00	64.73	69.25	67.44	1.73	1.07	27.08	0.036

4.8 Optimisation 3D Graph Responses

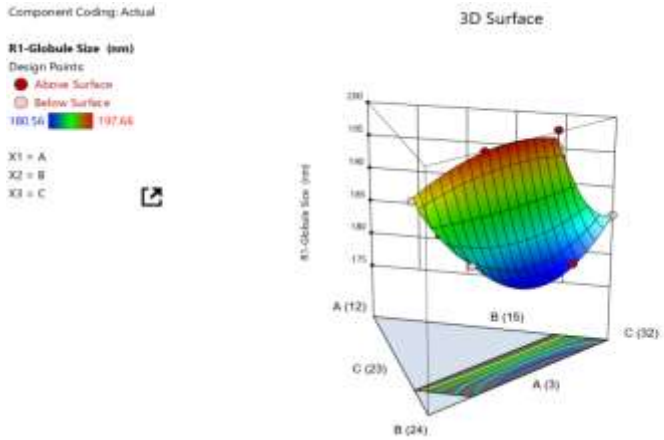


Fig. 10A: 3D Surface of R1 Globule Size

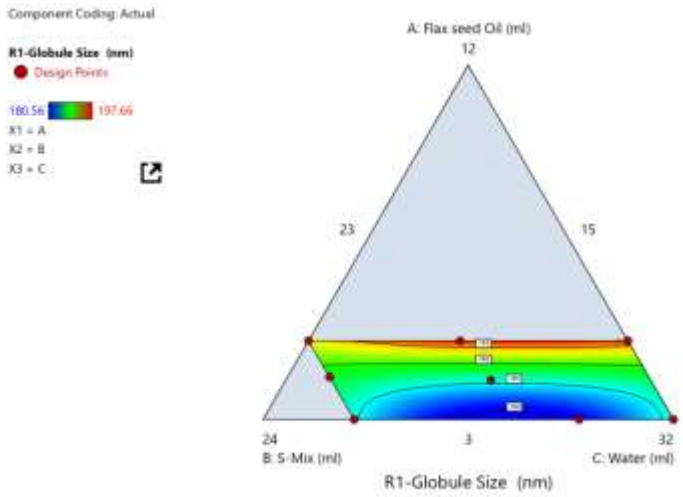


Fig. 10B: 3D Surface of R1 Globule Size

R1 Globule Size Interpretation: The Model F-value of 28.30 implies the model is significant. P< 0.05 indicates significant. P>0.1 indicates not significant. The Lack of Fit F-value of 0.23 implies not significant. Non-significant lack of fit is good.

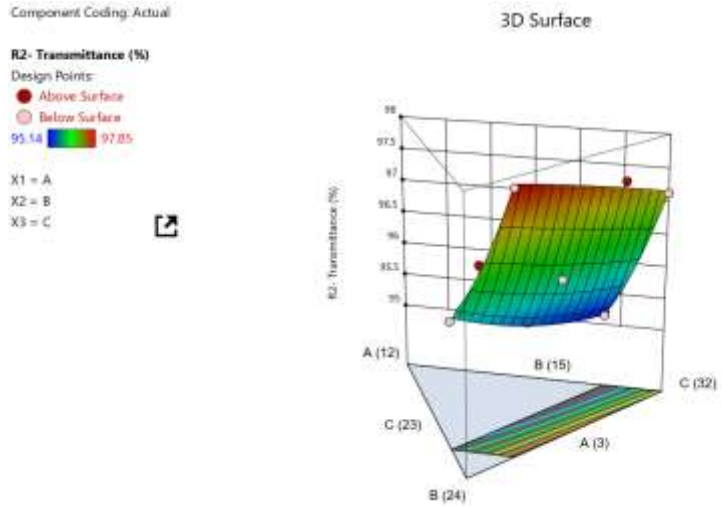


Fig. 11A: Transmittance (%)

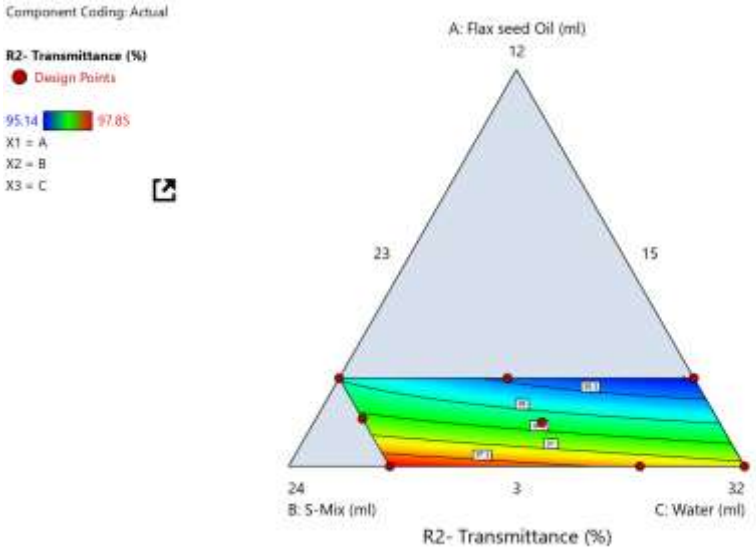


Fig. 11B: Transmittance (%)

R2 Interpretation: The Model F-value of 53.22 implies significant. The Lack of Fit F-value of 39.35 implies not significant.

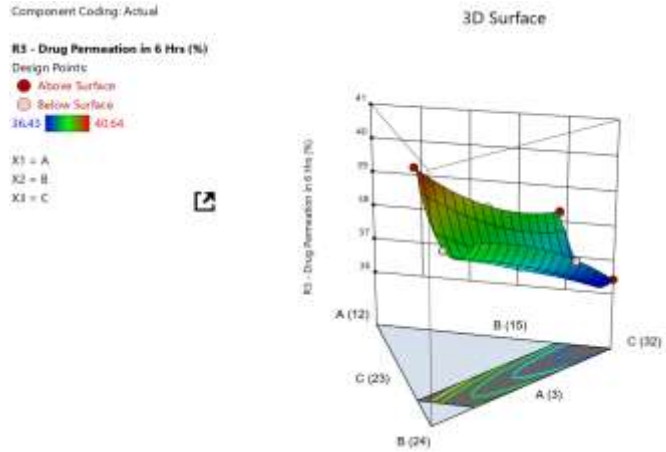


Fig. 12A: Drug Permeation in 6hrs. (%)

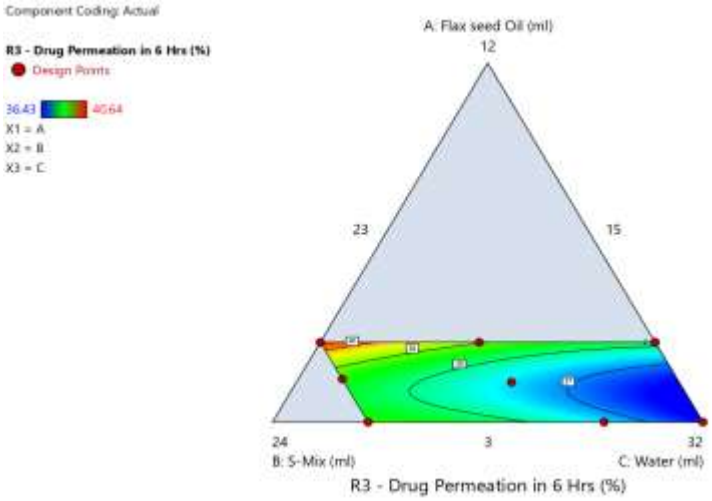


Fig. 12B: Drug Permeation in 6hrs. (%)

R3 Interpretation: The Model F-value of 486.45 implies significant. The Lack of Fit F-value of 3.37 implies not significant.

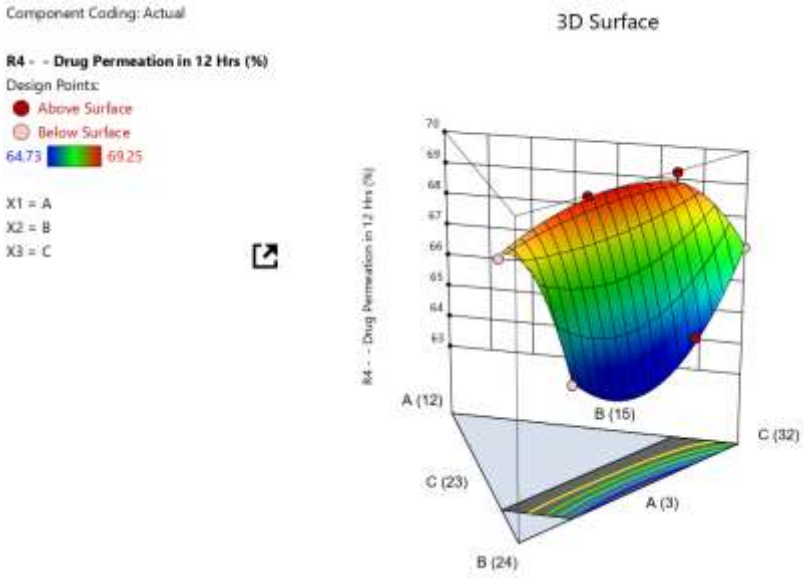


Fig. 13A: Drug Permeation in 12hrs. (%)

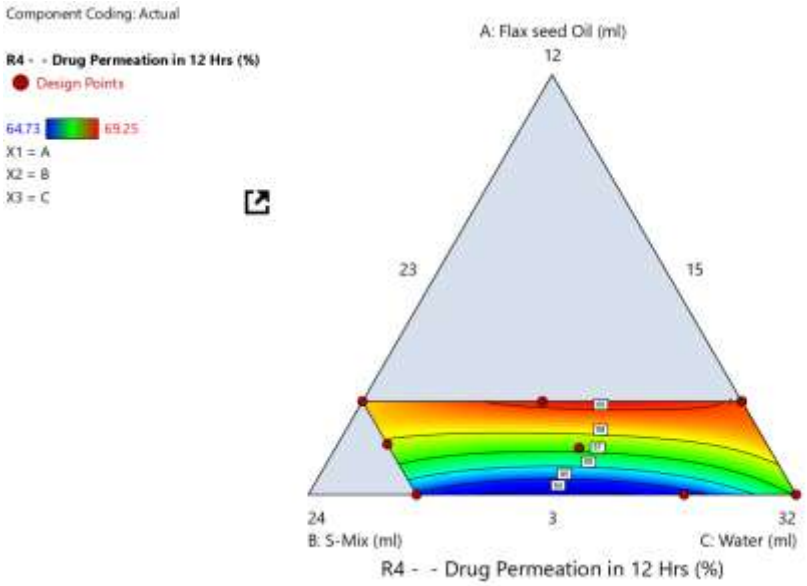


Fig. 13B: Drug Permeation in 12hrs. (%)

R4 Interpretation: The Model F-value of 27.08 implies the model is significant. The Lack of Fit F-value of 0.85 implies not significant.

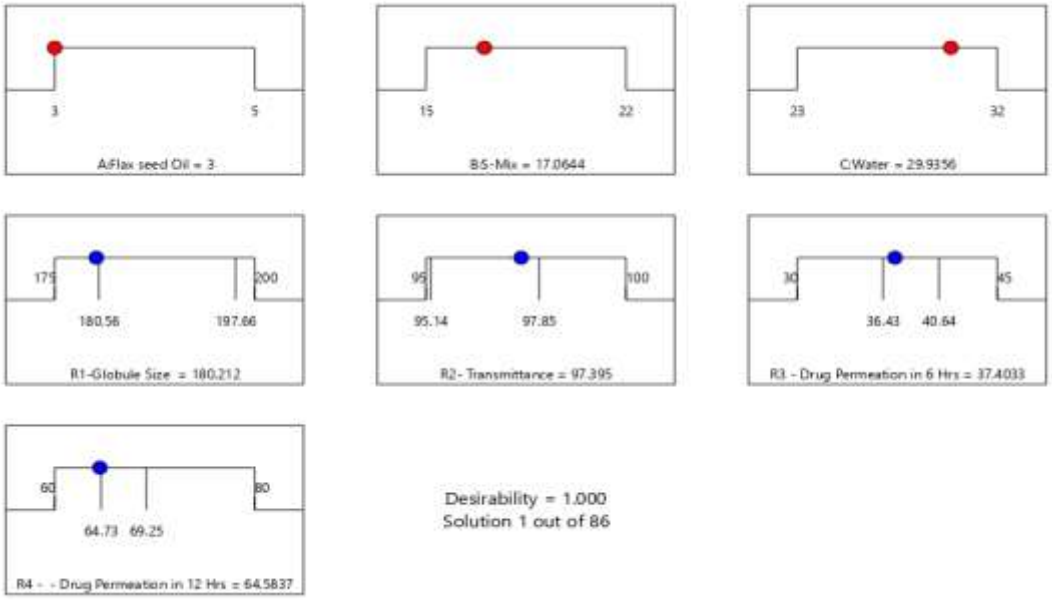


Fig. 14: Optimization Desirability Graph

Table 06: Coefficient Table

	A	B	C	AB	AC	BC	ABC
R1-Globule Size	465.819	194.995	185.963	-357.276	-299.186	-44.2557	268.669
p-values				0.0751	0.0973	0.0624	0.0686
R2- Transmittance	99.9059	98.0859	97.1563	-14.587	-15.0846	0.144202	-5.09512
p-values				0.3605	0.3367	0.9266	0.6233
R3 - Drug Permeation in 6 Hrs	106.354	38.9639	36.4173	**-77.067**	**-79.1894**	2.27378	**-23.3361**
p-values				**0.0040**	**0.0036**	0.0533	**0.0217**
R4 - - Drug Permeation in 12 Hrs	51.8706	67.4241	67.0996	24.9915	30.1813	**-14.654**	83.3725
p-values				0.4890	0.4053	**0.0477**	0.0592

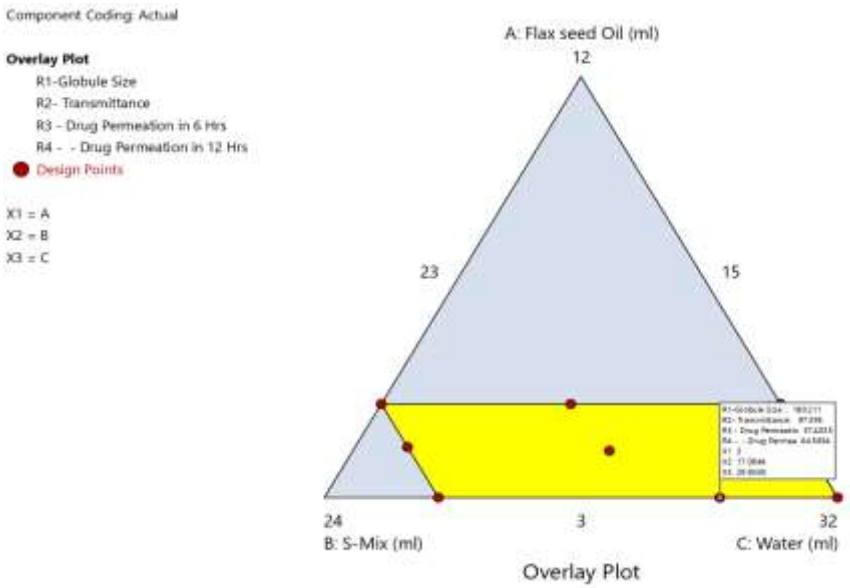


Fig. 15: Overlay Plot

4.9 Factorial Design for Ionotophoretic effect

Table 07: Build Information

File Version	13.0.5.0		
Study Type	Factorial	**Subtype**	Randomized
Design Type	2 Level Factorial	**Runs**	7.00
Design Model	2FI	**Blocks**	No Blocks
Center Points	3.00	**Build Time (ms)**	2.00

Table 08: Factorial Design for Ionotophoretic effect of Paroxetine Microemulsion

Std	Run	Factor 1	Factor 2	Response 1	Response 2	Response 3
		A:Current Density	B:Time of Current Application	Cmax	Tmax	Drug Content
		mA	Hr	%	Min	%
1	2	0	0	38.24	360	97.4
2	1	3	0	70.15	300	97.34
3	7	0	3	38.35	360	97.5
4	3	3	3	69.22	300	97.3
5	5	1.5	1.5	71.65	210	97.38
6	4	1.5	1.5	71.61	200	97.4
7	6	1.5	1.5	71.58	190	97.4

Table 9: ANOVA for selected Factorial Model

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio	F-value	p-value
R1	Cmax	%	7.00	38.24	71.65	61.54	15.91	1.87	2.664E+05	< 0.0001
R2	Tmax	Min	7.00	190	360	274.29	73.90	1.89	27.00	0.0121

R3	Drug Content	%	7.00	97.3	97.5	97.39	0.0620	1.00	56.75	0.0174

Interpretation:
Response R1: The Model F-value of 266424.49 implies the model is significant. Response R2: The Model F-value of 27.00 implies the model is significant. Response R3: The Model F-value of 56.75 implies the model is significant. P< 0.05 indicates significant.

4.10 Optimization of 3D Surface

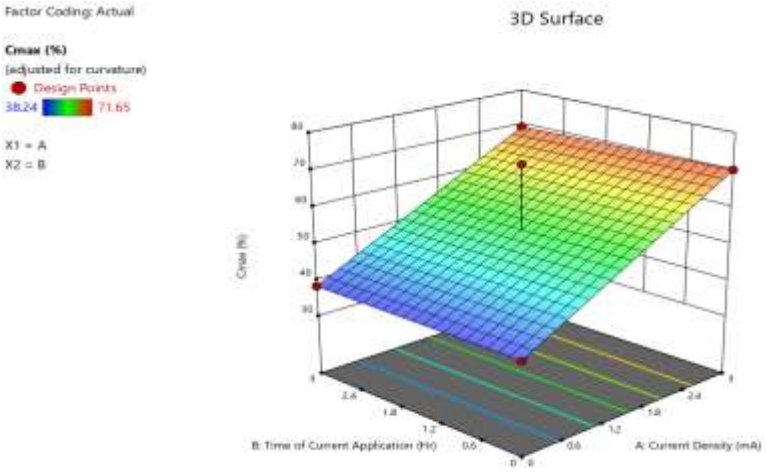


Fig. 16: Cmax (%)

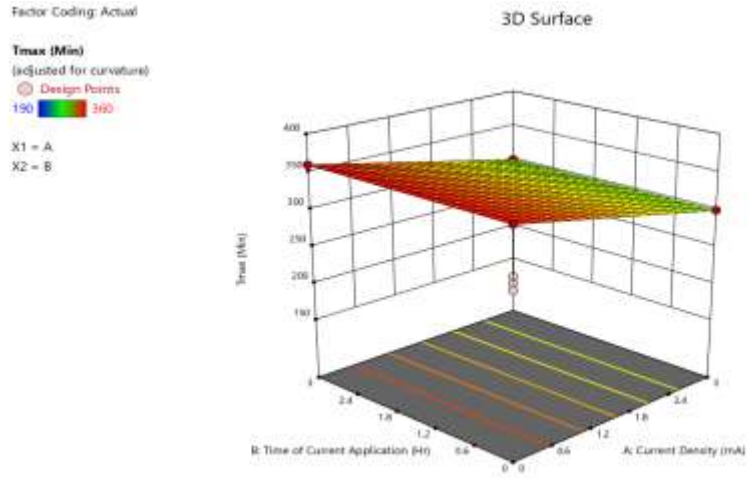


Fig. 17: Tmax (Min)

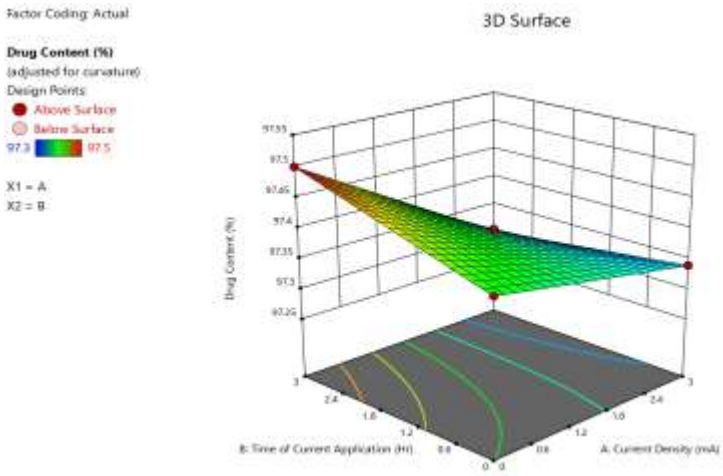


Fig. 18: Drug Content (%)

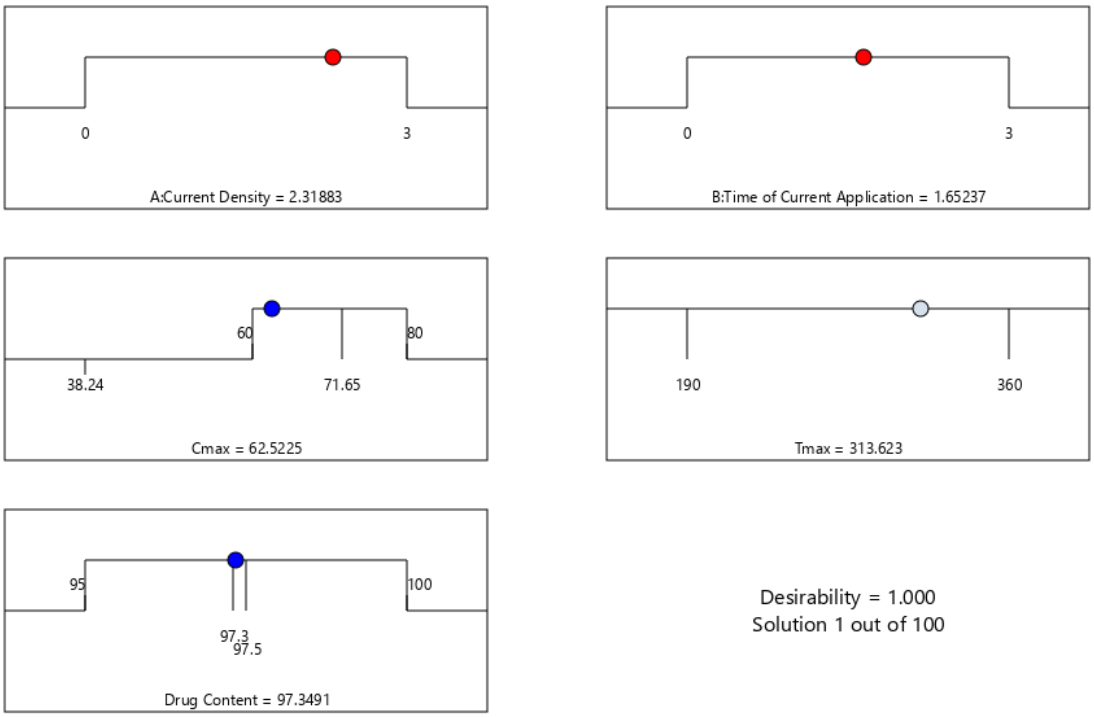


Fig. 19 Optimization Desirability Graph

Table 10: Coefficient Table

	Intercept	**A**	**B**	**AB**
Cmax	53.99	**15.695**	**-0.205**	**-0.26**
p-values		**< 0.0001**	**0.0073**	**0.0045**
Tmax	330	**-30**	-6.579E-14	
p-values		**0.0052**	1.0000	
Drug Content	97.385	**-0.065**	0.015	**-0.035**
p-values		**0.0078**	0.1217	**0.0261**

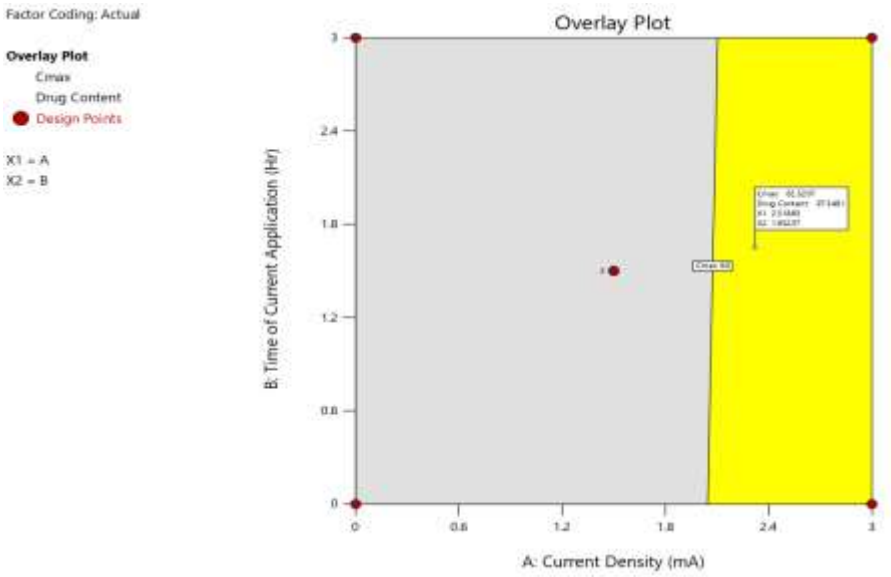


Fig. 20 Overlay Plot Graph

4.11 Observation of Microemulsion by Optical Microscope

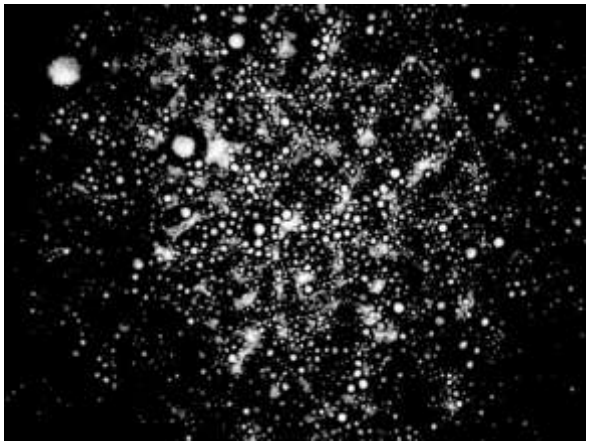


Figure 21B Globule size of microemulsion Flaxseed oil

Figure 21B Globule size of microemulsion Flaxseed oil

Fig. 21: Optical microscope of globule size of microemulsion of Flaxseed oil

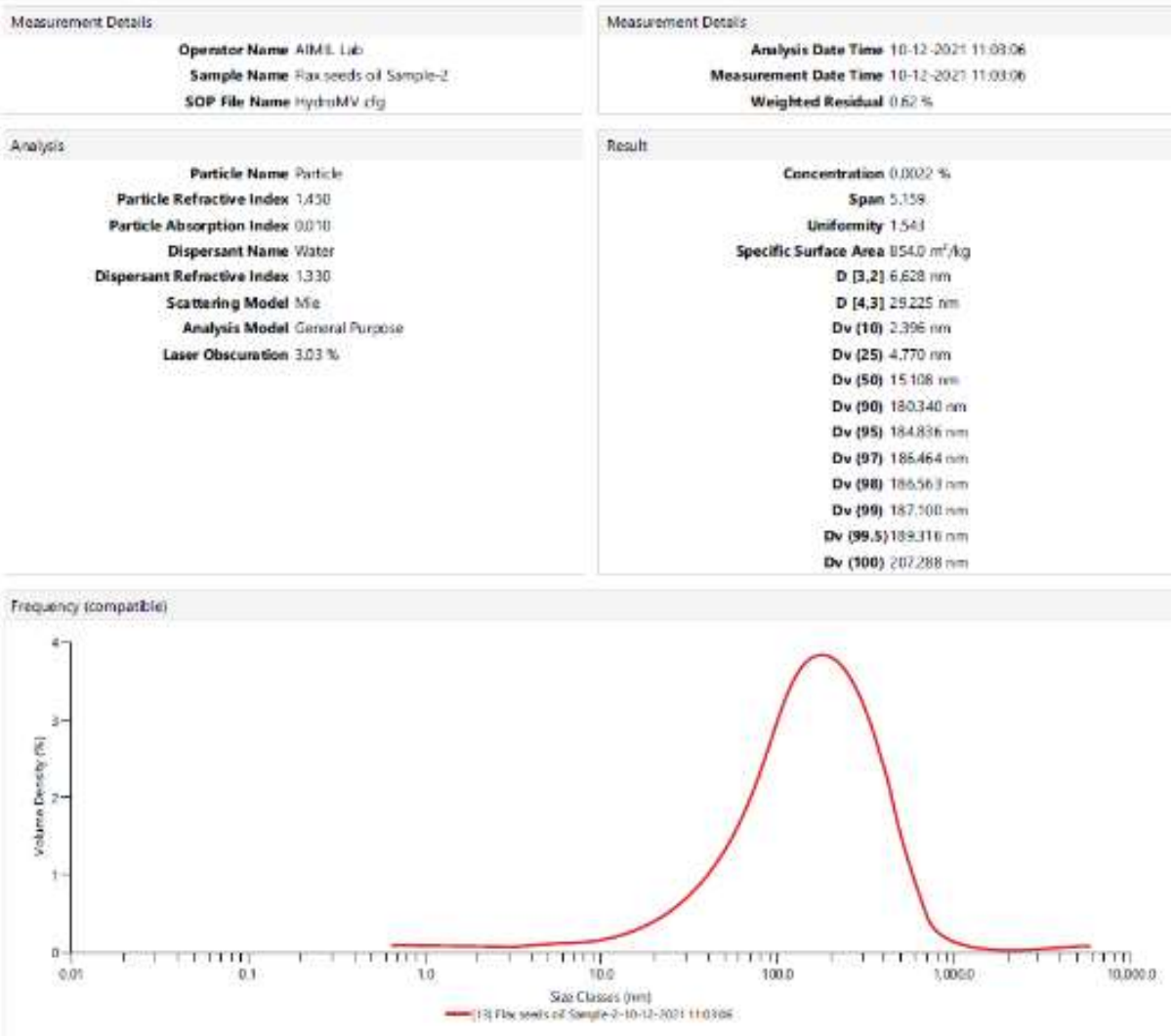


Fig. 22: Particle size distribution of Paroxetine HCl based Flaxseed oil microemulsion

Particle size distribution analysis report indicated that 50% of the particles are of 28.935µm and 90% of the particles are of 68.684 µm. Particles of 4.3 – 143 µm in size were observed in the sample (Fig. 22).

Sample Details

Sample Name:	Flax seeds oil Sample-2. 3		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	Karnataka College of Pharm...	**Dispersant Name:**	Water
Record Number:	231	**Dispersant RI:**	1.330
Date and Time:	10 December 2021 11:06:21	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	543.6	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	5

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-15.34	**Peak 1:**	-15.34	100.0	3.80
Zeta Deviation (mV):	3.80	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0679	**Peak 3:**	0.00	0.0	0.00
Result quality	Good				

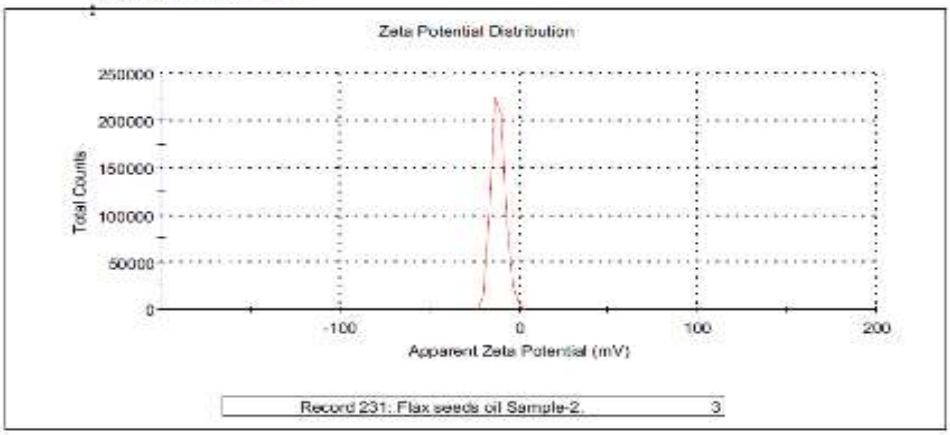


Fig. 23: Determination of Zeta potential

4.12 Differential scanning Colorimetry (DSC)

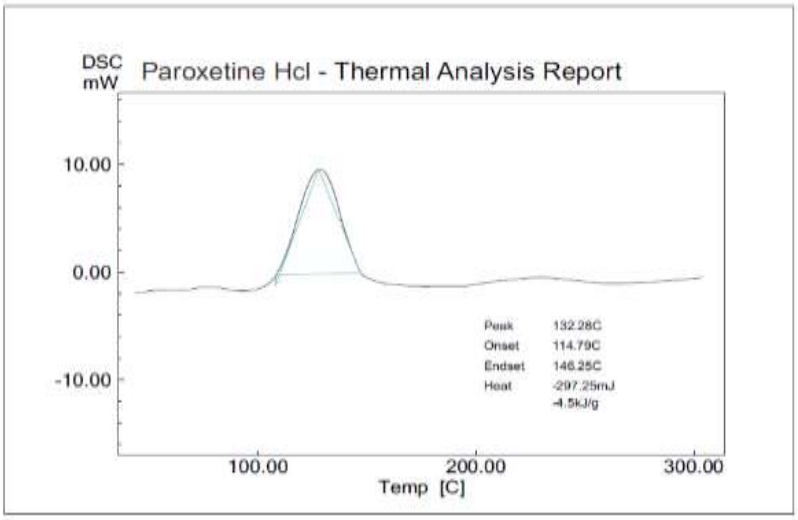


Fig. 24: Paroxetine HCl –Thermal analysis report

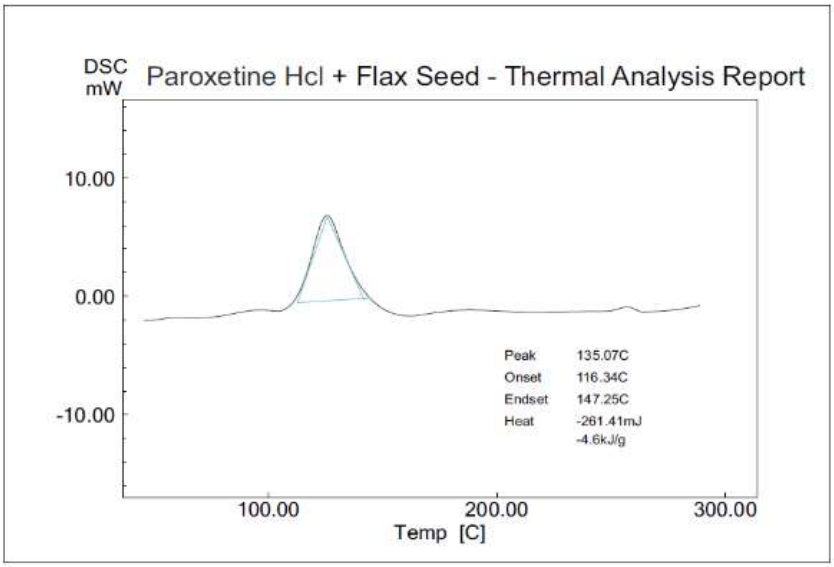


Fig.25: Paroxetine HCl Flaxseed Oil Microemulsion–Thermal analysis report

4.13 In-Vitro Diffusion Study of Optimised Formula

Table 11: In vitro release study, Zero order release kinetic, First order release kinetic, Higuchi model release kinetic and Korsmeyer-Peppas model release kinetic of optimized formula.

Time (hr)	% CDR	Log % rem	SQRT	Log T	Log % CDR
0	0	0	0	0	0
1	4.20042	1.981364	1	0	0
2	7.467833	1.966293	1.414214	0.30103	0.30103
3	15.60273	1.926328	1.732051	0.477121	0.477121
4	22.53831	1.889087	2	0.60206	0.60206
5	26.67431	1.865256	2.236068	0.69897	0.69897
6	34.8778	1.813729	2.44949	0.778151	0.778151
7	52.94976	1.672562	2.645751	0.845098	0.845098
8	60.82251	1.593037	2.828427	0.90309	0.90309

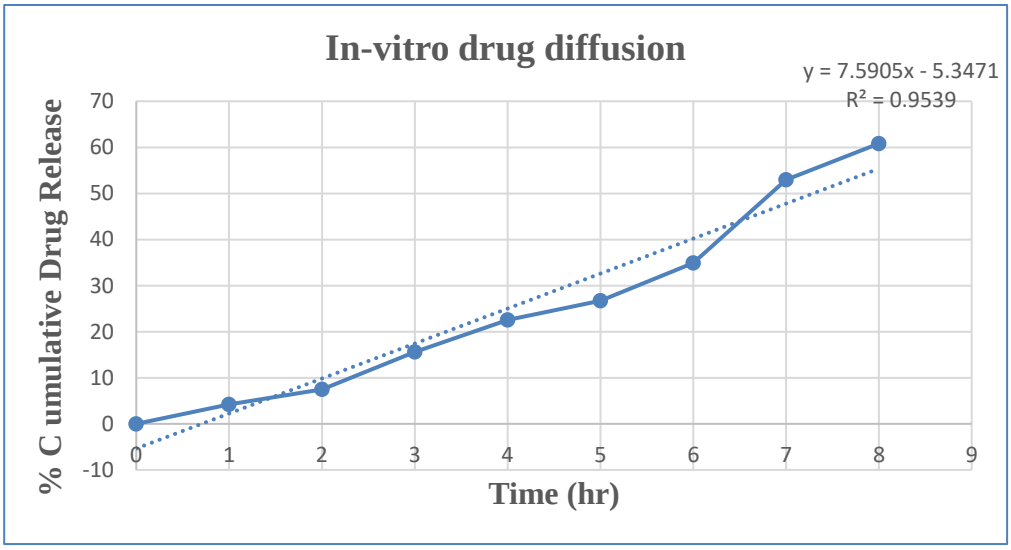


Fig. 26: *In-Vitro* plot of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

Fig. 27: First order plot of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

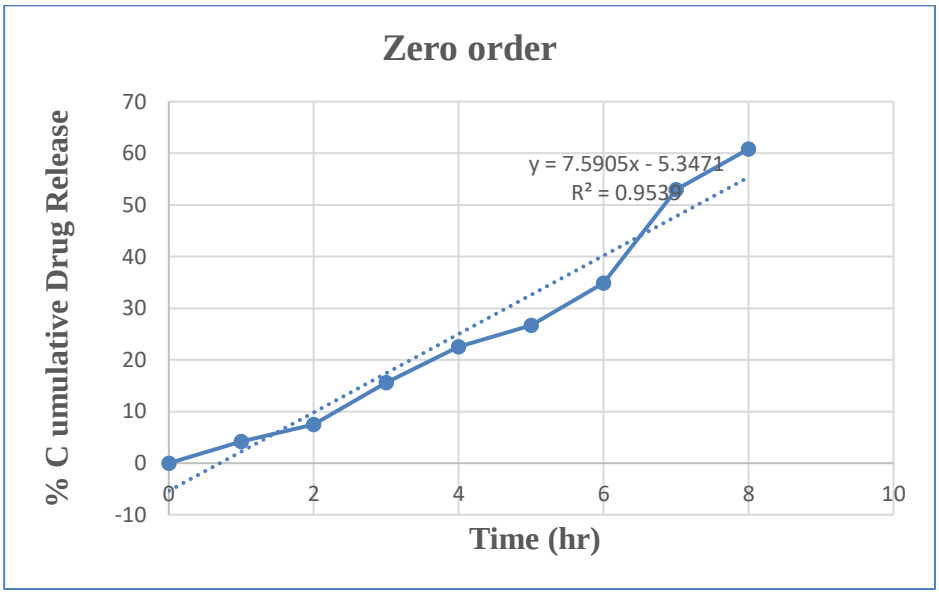


Fig. 28: Zero order of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

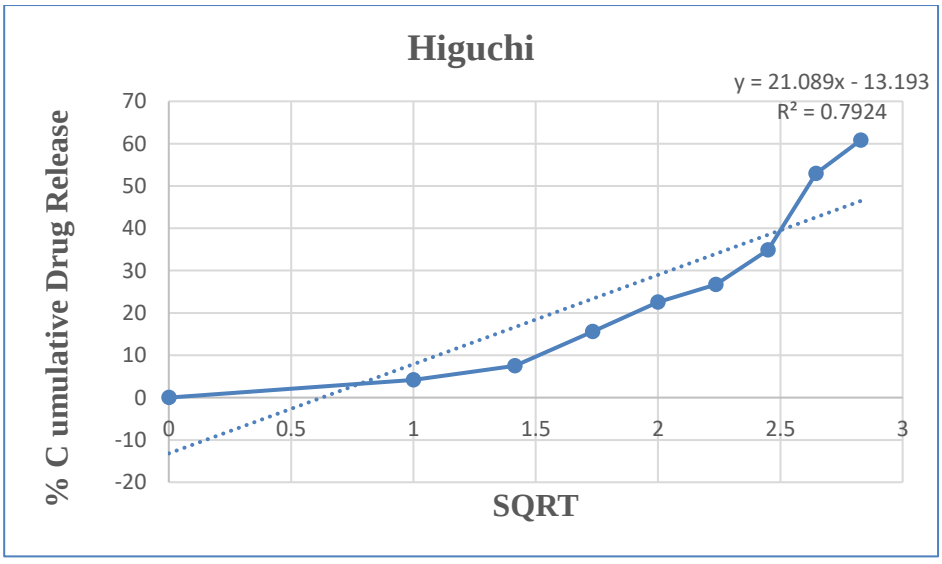


Fig. 29: Higuchi plot of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

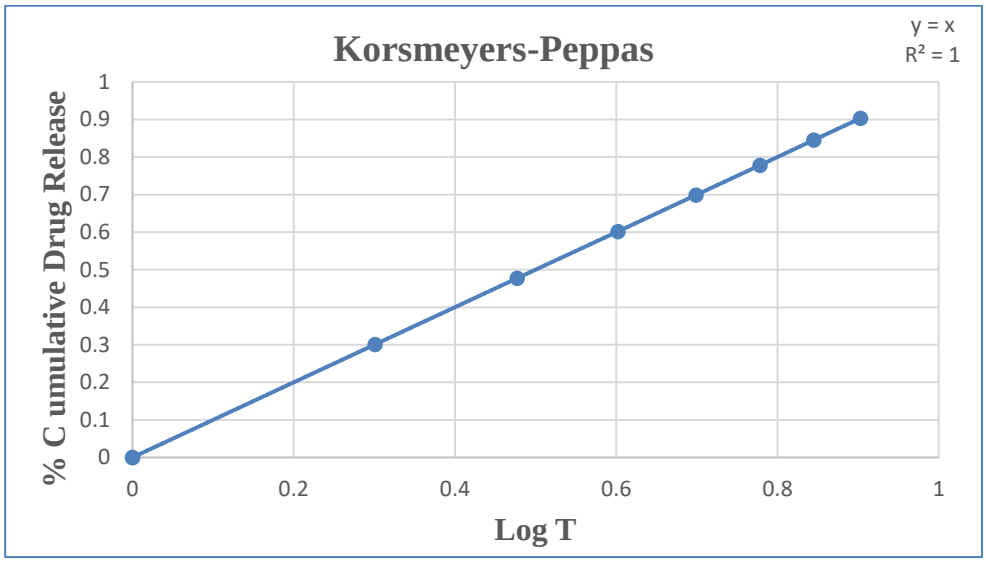


Fig. 30: Korsemeyer-peppas equation of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

4.14 Ex-Vivo Transdermal Permeation Study

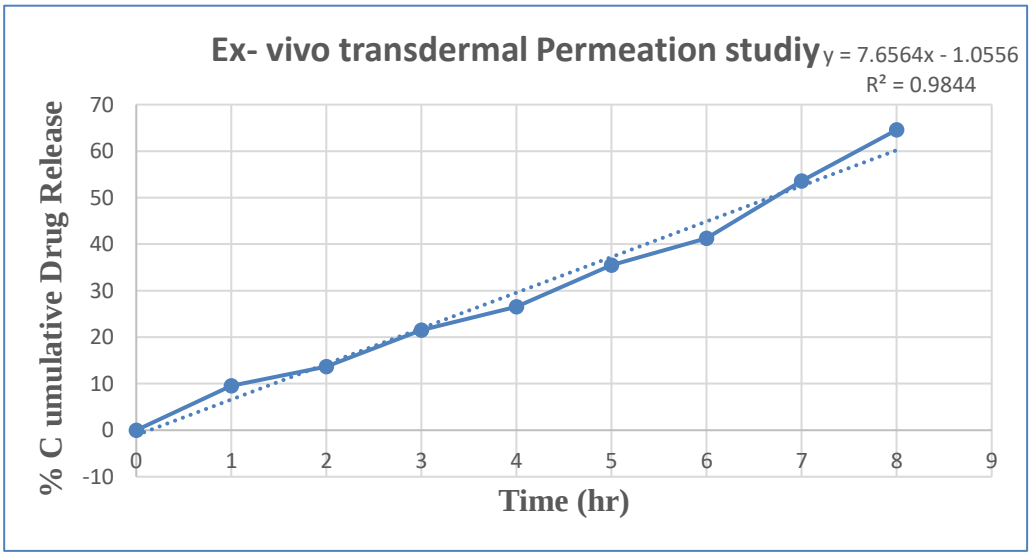


Figure 31: *Ex-Vivo* Transdermal Permeation Study of Optimized formula

4.15 Accelerated Stability Studies: Optimised Paroxetine HCl based Flaxseed Microemulsion Transdermal Gel

Table 12: Stability studies of Paroxetine HCl based Microemulsion Transdermal gel

Physicochemical Properties of Optimised formulation		**Days**		
		30	**60**	**90**
% Transmittance	**A**	97.89 ± 2.54	95.05 ± 2.39	91.75 ± 1.52
% Cmax	**A**	65.44 ± 1.08	62.29 ± 1.07	59.24 ± 1.69
Tmax (min)	**A**	326 ± 1.44	265 ± 2.05	224 ± 3.16
% Drug Content Uniformity	**A**	97.07 ± 2.07	95.59 ± 2.91	94.91 ± 2.65
% Cumulative drug diffusion	**A**	71.24 ± 2.09	69.97 ± 1.35	64.29 ± 1.12
% Cumulative drug Release	**A**	69.37 ± 2.45	63.04 ± 2.74	65.12 ± 2.26

Where A: 40ºc±2ºc/75%± 5%RH, n=3 (Mean ± SD

DISCUSSION

Topical formulations are good as the other dosage form because of their local effects and it happens through continence of medicine permeation to the layer of skin. The advantages of this formulation are as it has capability in accordance with deliver the drugs more selectively in imitation of a particular site. It gives the fast biological half-life, and increase the period of action.[22] Approximately 40% concerning new chemical entities show off poor aqueous solubility and provides a major role to current medicine delivery system as leads in imitation of poor absorption, less bioavailability, and lack of dose proportionality. However, in many instances, oral regimen is inept so the medicine undergoes significant degradation in the GIT or is metabolized to a high dimensions thru the first pass effect in the hepatic. These negative consequences intensified the inquire for an alternative drug delivery in the formulation of microemulsion-based hydrogel for local delivery.[23] The current drug molecule for the study was Paroxetine HCl, which is an antidepressant agent. The research demonstrated that the microemulsion formulation may stand to improve the solubility and skin permeability of drug called Paroxetine HCl. In the current study, an attempt has been made to formulate Paroxetine microemulsion based gel where different concentration of Oil %, S-mix % and Water % were used in different combinations. The prepared Paroxetine microemulsion was systematically subjected to evaluation and characterization. It has found that the estimation of Paroxetine by UV-Spectrophotometric method at λmax 271 nm which is shown in fig.03. Preformulation studies were carried out by mixing the drug with various excipients in different proportions, no significant change appear in the sample. No incompatibilities were observed between drug and excipients which was carried out by FT-IR (fig. 07-09). Solubility studies were obtained in different oils, surfactants and co-surfactants and the oils, surfactant and co-surfactant which show more solubility are selected for the preparation of microemulsion (Fig. 05). The optimization of Paroxetine microemulsions was done by d-optimal design; where % Oil, % S-mix and % Water were taken as variables and Transmittance, and %Drug release as a response (Table 04 and 05). The optimised Paroxetine microemulsions formulations were fabricated subjected to perform a 2^2 factorial design (Table 08 and 09). Pseudo ternary phase diagrams have been constructed to identify the microemulsion region and to optimize the concentration of oil, surfactant & co-surfactant. From the optimization, the desirability of different variables was obtained and thus optimized formula was obtained. The ANOVA and regression analysis were demonstrates that the formulation was significant for all response variables. The formulations were subjected to different thermodynamic stability stress tests like heating cooling cycle, centrifugation and freeze thaw stress tests. And the results were shown in Table 02, it was found that all the formulations were stable in all tests and selected for further characterization and evaluation. The clarity of microemulsions was checked by transparency, measured in terms of % T. The % transmittance was found in the range of 95.14% - 97.85%. The particle sizes of microemulsion were employed by using Malvern instrument. The samples were loaded onto 1cm2 cuvette in a thermo stated chamber. For microemulsion particles size ≤100 nm. It was reported that the smaller particle size gets more absorption and bioavailability. The increased absorption of microemulsion can be attributed to nano sized fine droplets as observed in droplet size analysis. This increased surface area has direct impact on the improved contact with intestinal mucosa and thus better absorption. This is clearly indicated in reduced Tmax for the microemulsion (Fig. 21). Particles with zeta potentials more positive and negative +30mV and -30 mV are normally considered stable. The optimal batch of zeta potential is -15.34 mV, which shows this is considerable as stable product (Fig. 22 and 23). DSC was found to be in fairly acceptable (Fig. 24 and 25). The % Drug content of optimized formulation was found to be 97.39%, which complies with pharmacopeial specifications (Table 08 and 09). In vitro drug diffusion results indicate complete diffusion of drug from all its microemulsion within 1 to 8 hour which is depicted in Table no.11. The in-vitro drug diffusion of optimized formulation was found to be 60.82% which shows deviated results than the result reported in the reference article. The release studies of the formulation were analyzed on the basis of zero order, First order, and Korsmeyer Peppas kinetics (Fig. 26 to 30). The *Ex-vivo* drug release studies have shown the drug release for the optimized formulation across the porcine skin model. The maximum drug release was found to be 64.57% at 8hr (Fig. 31). Accelerated stability studies were performed as per the ICH guideline over a period of 3 months. The results of the studies tabulated in Table 12 show that the microemulsion did not change significantly in the physical appearance, assay and % CDR. Therefore we can conclude that the formulation is stable.

CONCLUSION

The current study has been a satisfactory attempt to formulate a Paroxetine HCl microemulsion for transdermal gel. From the experiments, it can be concluded that, microemulsion of Paroxetine HCL were prepared using Flaxseed Oil (3ml), surfactant mixture consisted of Tween®80 & Propylene glycol in 1:3 ratio (17.06ml) and double distilled water (29.93ml) as an aqueous phase. The FTIR was no interaction between excipients; they are compatible with each other. PDI and Zeta potential was measured and the mean particle size and distribution of microparticles was in the range, % Transmittance, Cmax, Tmax, drug content and % CDR were found to be fairly acceptable range. Optical microscope was observed global size of microemulsion studies indicate surface topography having round surface of the formulation, DSC were recorded to see the drug status. In-vitro and ex-vivo shows a significant effect on drug release. Stability studies revealed that optimized formulation was stable. Finally it was concluded that the prepared Flaxseed oil microemulsion of Paroxetine HCl transdermal gel may prove to be potential enough for effective drug delivery.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS

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