Original Article

"Development And Optimization Of Paroxetine Hydrochloride Loaded Microemulsion Almond Oil Based Transdermal Drug Delivery"

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Abstract

Background: Paroxetine HCl is the antidepressant drug which is known to be a most potent serotonin reuptake blocker. This study was aimed to reduce the consequences accompanied with the fluctuations in concentration of plasma level after oral administration of drug and additionally in imitation to minimize broad metabolism of the same in the hepatic by flourishing and optimizing the formulation of transdermal paroxetine hydrochloride in order to increase its bioavailability and safety. **Objectives:** In the present research, paroxetine Hydrochloride microemulsion transdermal gel as alternative route of drug administration have been prepared and characterized. **Methods:** A pseudo-ternary phase diagram, D optimal and Factorial designs have been used to observe and optimize the microemulsion. Using pseudo-ternary phase diagram microemulsion area was subjected to select and according to that ratio of percentage oil, percentage surfactant and percentage co-surfactant, microemulsion were optimized. Based on phase diagrams, formulations have been prepared and elucidate for various parameters including compatibility test within the drug and excipient by FT-IR. The prepared transdermal gel was tests for analysis of size distribution, Zeta potential, thermal analysis, % CDR, % transmittance, globule size, Cmax, Tmax, drug content, *in-vitro* diffusion, *ex-vivo* permeation, and accelerated stability studies were performed. **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. 79.23% in 8h. The optimized formulation having the combination of Almond Oil (3ml), surfactant mixtures were consisted of Tween®80 & Propylene glycol in 1:3 ratio (15ml) and DDW (32ml) as an aqueous phase showed best physicochemical parameters with 89.12 % 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. **Conclusion:** The developed Paroxetine HCl microemulsion based transdermal gel showed good physicochemical properties. It is concluded from the present studies that Paroxetine HCl microemulsions shown a potential transdermal drug delivery system with virtuous stability and drug release profile.

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

INTRODUCTION

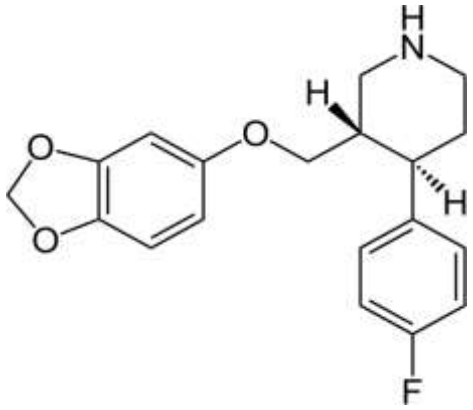
Depression is commonly characterised via emotions over sadness accompanied through impassioned and physical withdrawal, while anxiousness is a concern which is caused by administration not composite immediately in conformity with a discernable stimulus. Clinically, each consequence is treated with antidepressants drug and combined with non-pharmacological counseling, which may improve the patient compliance. However, not every patient respond in imitation of antidepressant treatment, the pharmacological treatment results take some weeks to get the respond and it consequences are frequently accompanied through the use of undesirable issue effects. The development of consequences needs more targeted and faster-acting remedies require the grasp over the mechanisms, which involved among the development over anxiousness and depression[1].

Transdermal drug delivery with having psychotropic properties permits mental wellbeing's developers to customise the remedy for patients by altering the period over therapy, minimizing the hepatic metabolism and avoid the drug–drug interactions, and

also reducing the risk of GIT irritation. Another potent skills on it mode of transport is the pleasure about use; patches can additionally lie utilized over the skin daily or once a 7days to avoid the administration of oral dosage preparations on daily basis[2].

A microemulsion is thermodynamically steady, transparent an isotropic, and translucent dictation of oil, water and surfactant in imitation of up to expectation amount is; often prepared of mixture including a co-surfactant to that amount has a 5-100 nm droplet size. Due in conformity with theirs tiny droplet size, microemulsions have been obtained activity for pharmaceutical purposes as like carrier systems for dermal/transdermal drug delivery due to the fact they grant several advantages on conventional periodic formulations certain namely creams, ointments, and gels.[3] There is a necessity for a remedy delivery provision that may enhance the bioavailability over certain drugs, who would keep associated with a change in the ability and tolerability of drug treatments and making sure of accelerated compliance including a decrease occurrence and an more desirable effect while administering the drugs with user friendly, convenient, painless, and provide improved patient compliance too. The objective of this study is to formulate a novel transdermal formulation of a Paroxetine Hydrochloride drug with modified drug release properties for site specific delivery.

1.1 Drug Profile of Paroxetine Hydrochloride[4]



Paroxetine Hydrochloride | C19H21ClFNO3

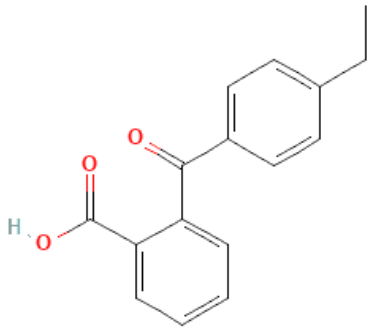
IUPAC Name: (3S, 4R)-3-[(2H-1, 3-benzodioxol-5-yloxy) methyl]-4-(4-Fluorophenyl) piperidine

1.2 EXCIPIENT PROFILE[5, 6]

Almond Oil, Prunus dulcis Oil (Common Name)

Formula and Molecular weight: C16H14O3 and 254.28

Str. Formula:



Properties:

The physicochemical properties of almond oil were: color (yellow), refractive index (1.465), specific gravity (0.981), acid value (1.3±0.4mgKOH/g, saponification value (128.0±0.3mg KOH/g), iodine value (65±0.1mg Iodine/g), peroxide value (2.8±0.2)

and free fatly acids (0.64±0.1mg/g). Boiling point: 430°F, Melting point: 134-136°C, Solubility: Insoluble in cold water, slightly soluble in hot water and ether, soluble in ethanol, Density: 1.179g/cm3.

MATERIALS AND METHOD

2.1 Materials and Reagent

Drug: Paroxetine HCL (Procured from: Yarrow Chemical Products, Mumbai, Maharashtra, India), **Oil:** Almond 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/cm³, and 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 -1700 instrument, shimadzu, 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 drug melting point was observed by using open capillary method.[7]

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.[8]

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 has been prepared by 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 blank (PBS, pH 7.4) using Shimadzu double beam UV-visible spectrophotometer 1900-I

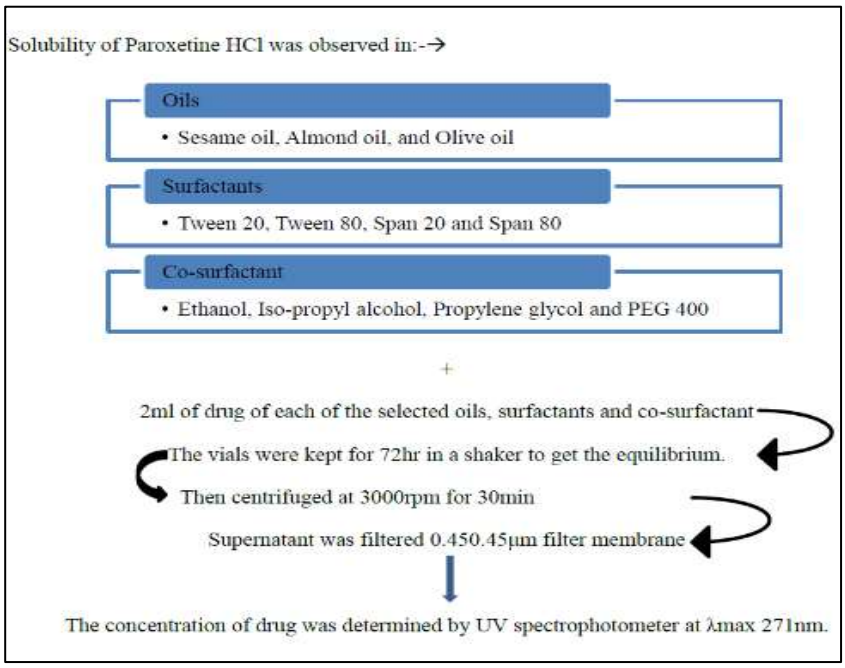
2.3.5 Standard stock solutions of Paroxetine HCl in Ethanol: Accurately weighed 100mg of standard Paroxetine HCl was dissolved in 100mL of Ethanol.

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 presented on Table 01.

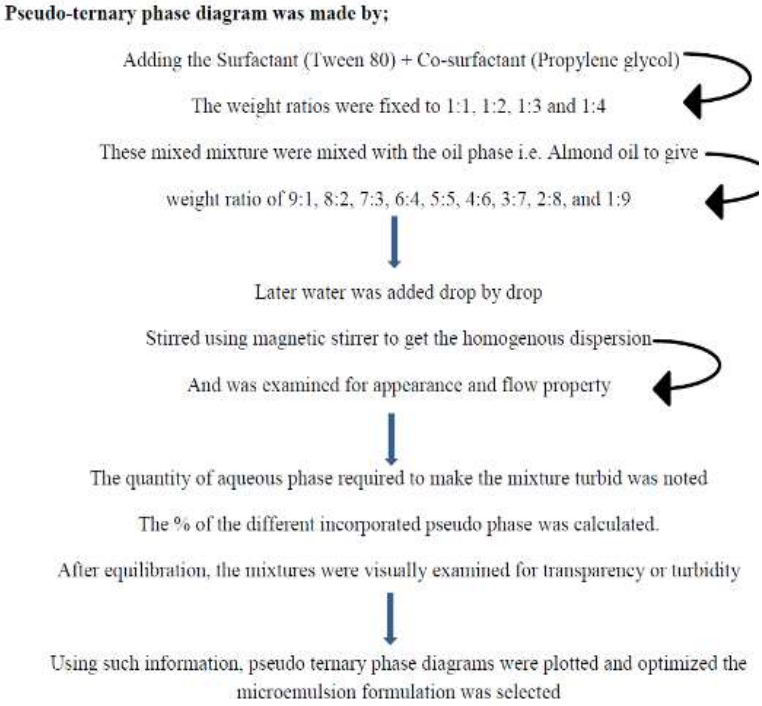
2.4 Pre-Formulation Compatibility Studies

2.4.1 Selection and Screening of Oils, Surfactants and Co-surfactant for Microemulsion components:[9]



2.5 Construction of pseudo-ternary phase diagrams

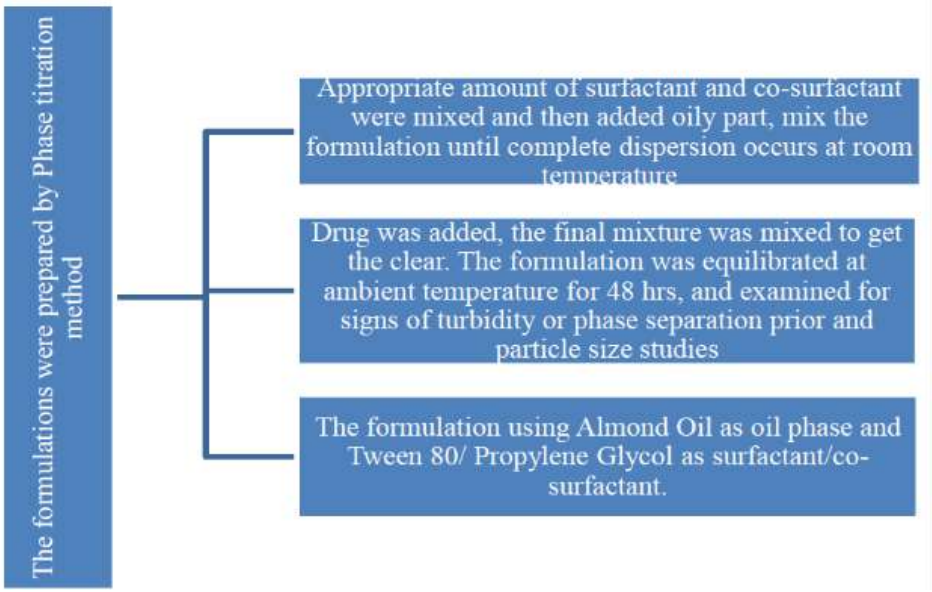
The Phase diagram was constructed by using Oil, Surfactant and H2O ratio with various combinations from the literature and solubility study to perform the Preformulation studies to formulate Paroxetine HCl microemulsions. The microemulsion existence region was employed by Titration method and constructed 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 almond oil microemulsions were prepared using D-Optimal Design. Design-Expert® software was used for analyzing the experimental design. 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 Almond 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® 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 gel11-16

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 was not showing 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 criteria, were taken for dispersibility test (Assessing the efficiency of self-emulsification).

2.9.2 Quantitative Test:

a) Percent 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 Almond 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
It was carried out by dilutions in MQ H_2O at 25.1°C for 70sec and used dynamic light scattering (Malvern ZS instrument)..

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 have been analyzed by using UV visible spectrophotometer at 271nm.

The results were plotted as CRD % vs. time**.**[17]

2.9.9 *Ex-Vivo* transdermal Permeation study
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.[17]

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

2.10 Accelerated Stability Studies

It was obtained 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.[18]

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 128°C.

4.2 The solubility

The solubility of the selected drug was obtained with various 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.

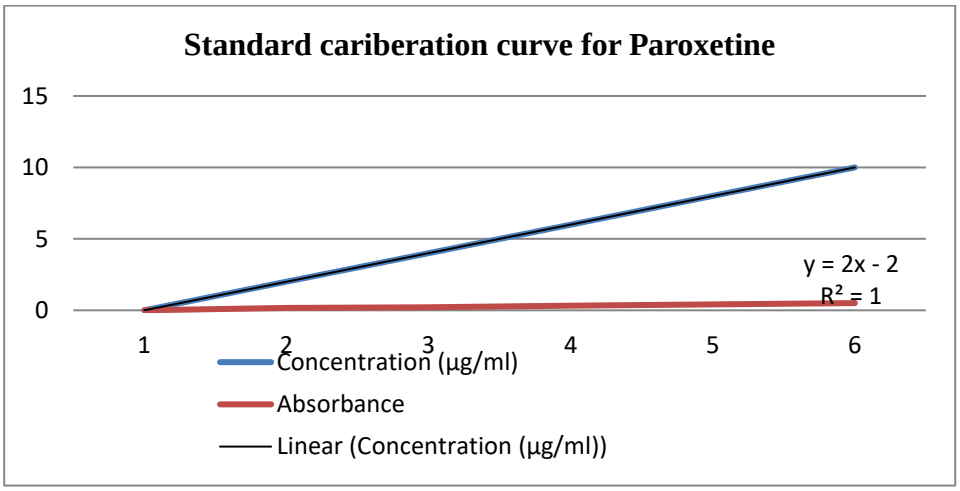


Fig.01: Std. Calibration curve Paroxetine HCl

4.3.2 Standard Calibration Curve for Paroxetine HCl in Ethanol

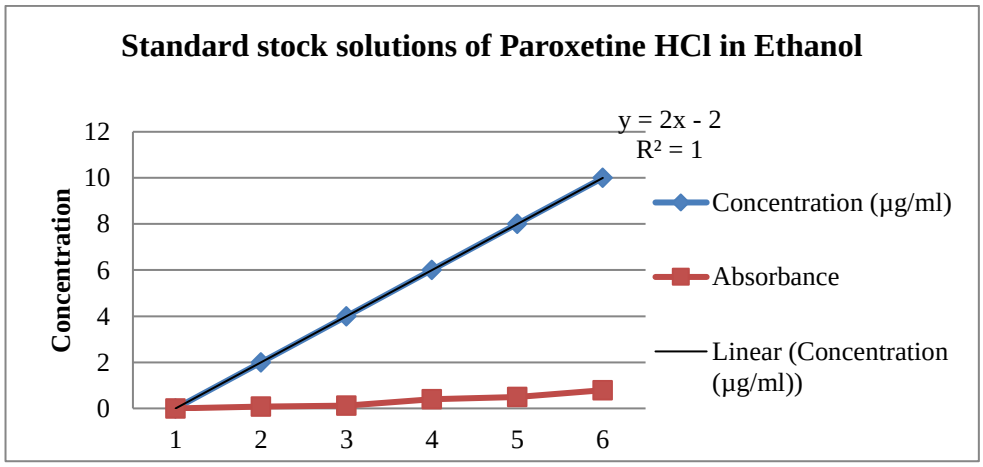


Fig. 02: Std. Stock Soln. of Paroxetine HCl in Ethanol

4.4 Identification of Drug (Paroxetine HCl) by FTIR Spectroscopy

The FTIR spectrum of Paroxetine Hcl is presented in Fig. 03 and its corresponding interpretation for identification of functional groups and bonds is given in Table 01.

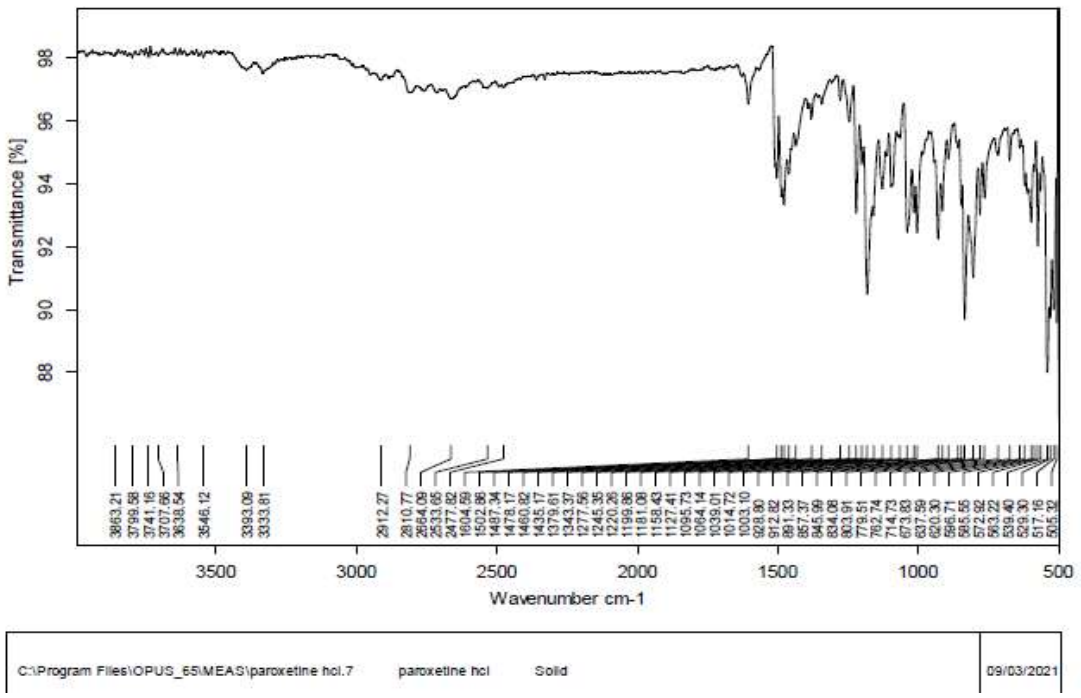


Fig. 03: FTIR Spectrum of Paroxetine HCl

4.5 Pre-Formulation Compatibility Studies

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

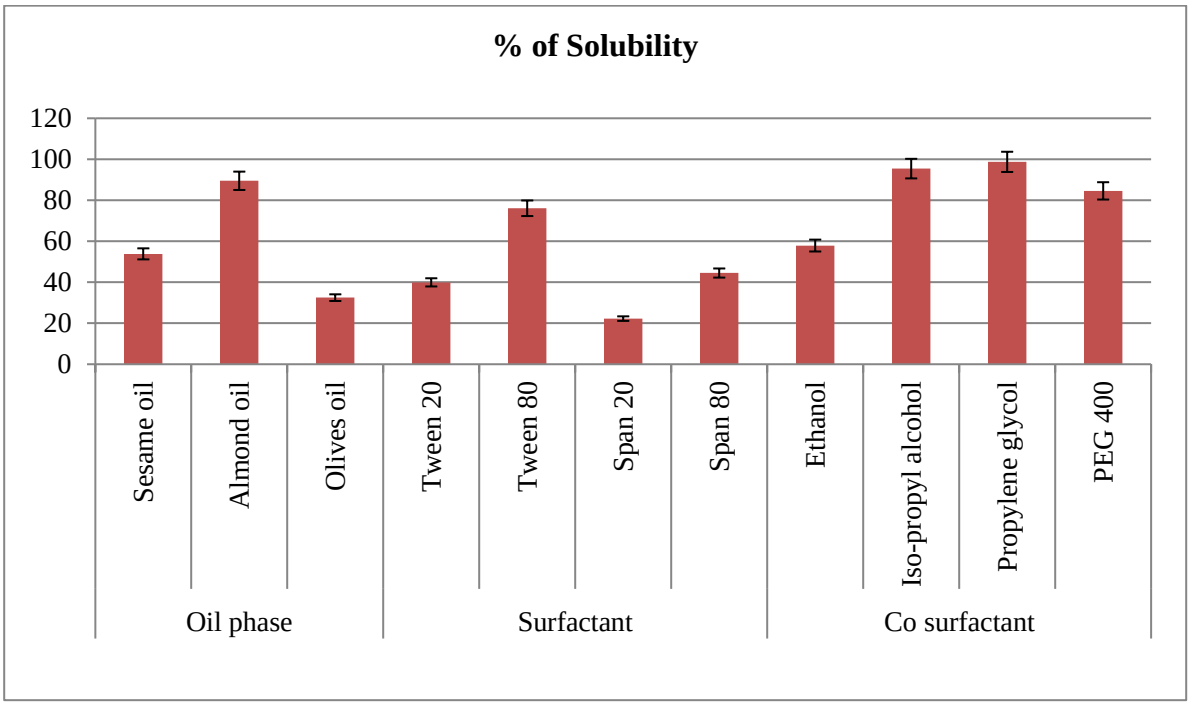


Fig. 04: Schematic diagram of drug solubility in different Oil, Surfactant, and Co-Surfactant

The Maximum solubility of drug candidate in Oil phase, Surfactant and co-Surfactant was found to be in Almond Oil (89.50%), 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

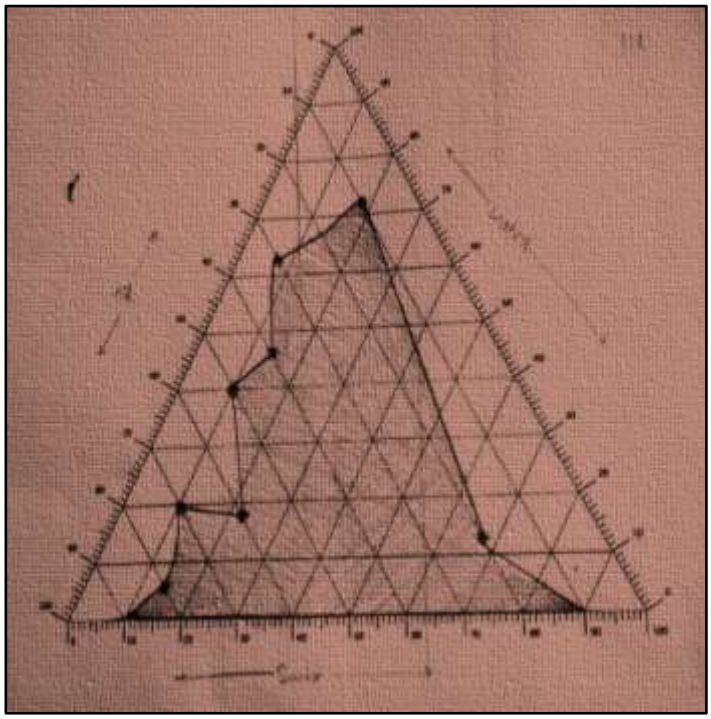


Fig. 05: Pseudo ternary phase diagrams for Microemulsion: Oil phase consisted of Almond Oil (3ml), surfactant mixture consisted of Tween®80 & Propylene glycol in 1:3 ratio (15ml) and double distilled water (32ml) as an aqueous phase. Dark shaded region indicated Microemulsion region.

4.6 Drug-Excipient Compatibility studies by FTIR:

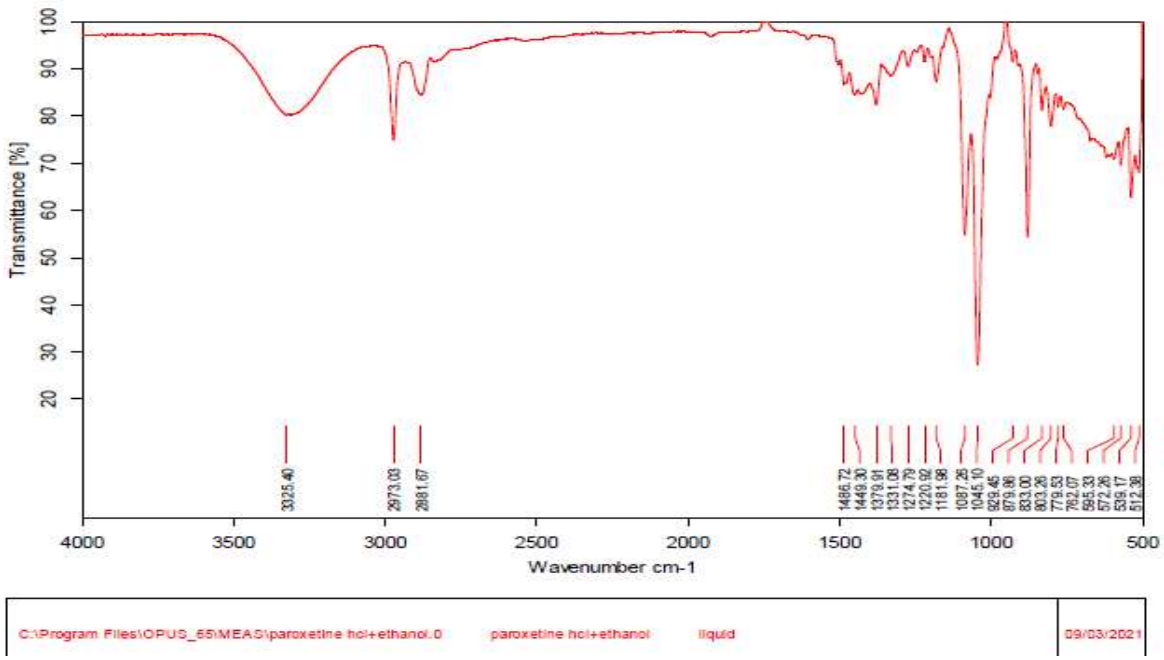


Fig. 06: FT-IR of Paroxetine with Ethanol

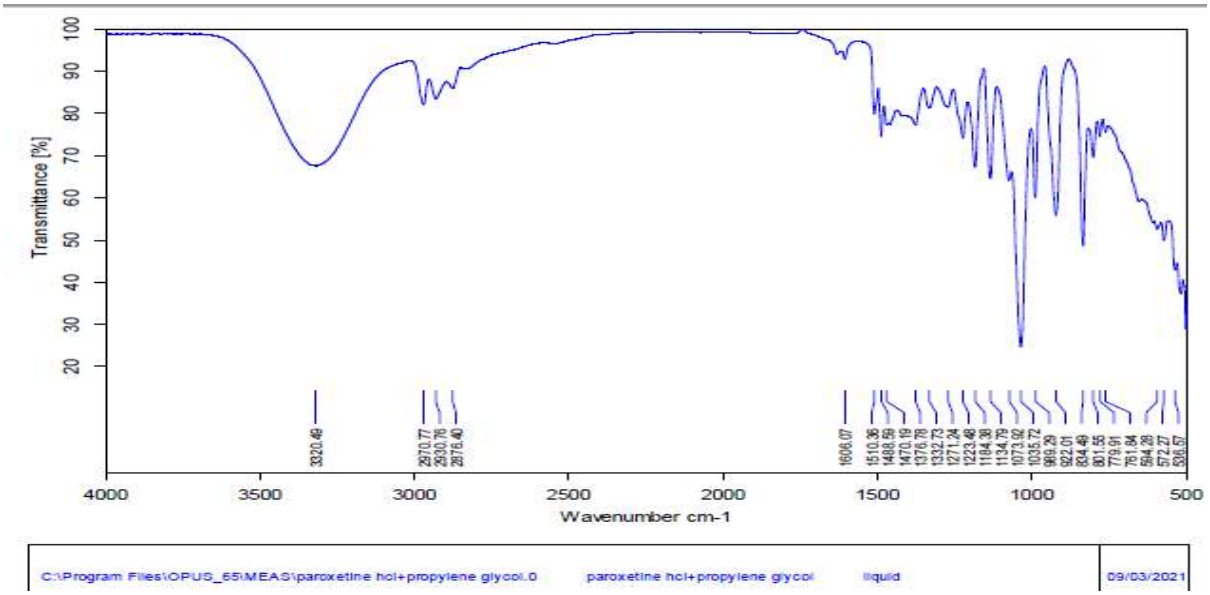


Fig. 07: FT-IR of Paroxetine with Propylene Glycol

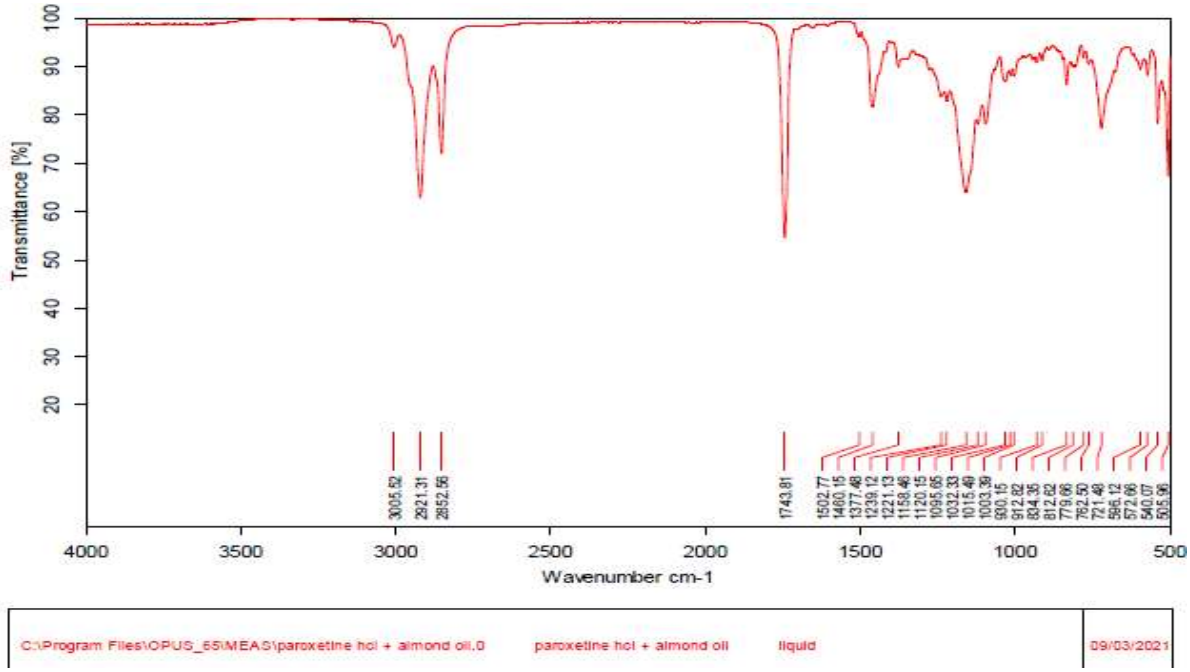


Fig. 08: FT-IR of Paroxetine with Almond Oil

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 + Almond Oil	-	2852.56	-	1032.33

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 Almond 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)	234.00		

Table 04: Characterization of Microemulsion by Factorial Model

	Component 1	Component 2	Component 3	Response 1	Response 2	Response 3	Response 4
Run	A:Almond 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	3	17.1312	29.8688	168.5	98.41	32.05	62.14
2	4	18.5	27.5	155.4	97.79	34.27	64.15
3	5	18.8049	26.1951	171.3	97.41	35.77	65.74
4	5	22	23	173.2	97.75	36.06	66.14
5	3	22	25	174.6	98.89	32.41	62.66
6	5	15	30	175.3	97.1	35.7	65.59
7	4.00058	22	23.9994	156.7	98.11	34.56	64.57
8	3	15	32	165.2	98.03	31.85	62.05
9	5	15	30	177.8	97.12	35.74	65.61

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	155.4	177.8	168.67	8.07	1.14	49.17	0.02
R2	R2- Transmittance	%	9.00	97.1	98.89	97.85	0.5914	1.02	3125.18	0.0003
R3	R3 - Drug Permeation in 6 Hrs	%	9.00	31.85	36.06	34.27	1.73	1.13	6423.84	0.0002

R4	R4 - - Drug Permeation in 12 Hrs	%	9.00	62.05	66.14	64.29	1.63	1.07	34884.77	< 0.0001

4.8 Optimisation 3D Graph Responses

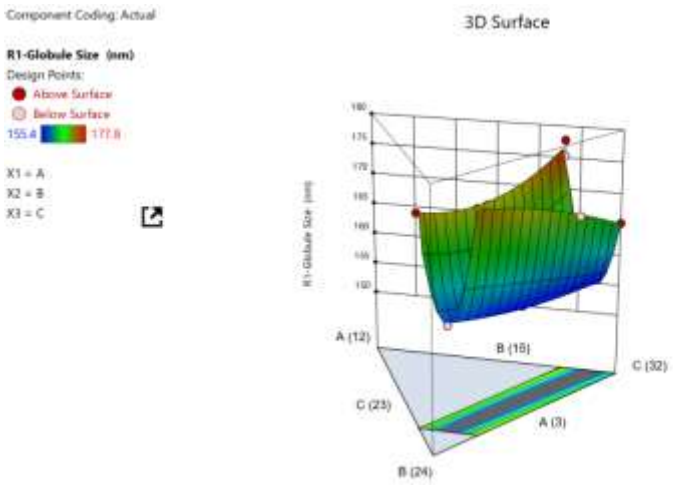


Fig. 09A: 3D Surface of R1 Globule Size

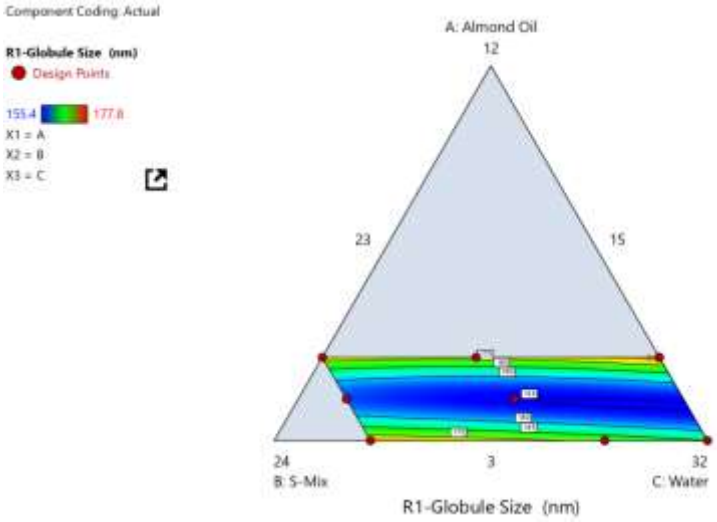


Fig. 09B: 3D Surface of R1 Globule Size

R1 Globule Size Interpretation: The Model F-value of 49.17 implies the model is significant. P< 0.05 indicates significant, P>0.1 indicates not significant. The Lack of Fit F-value of 0.12 implies not significant. Non-significant lack of fit is good.

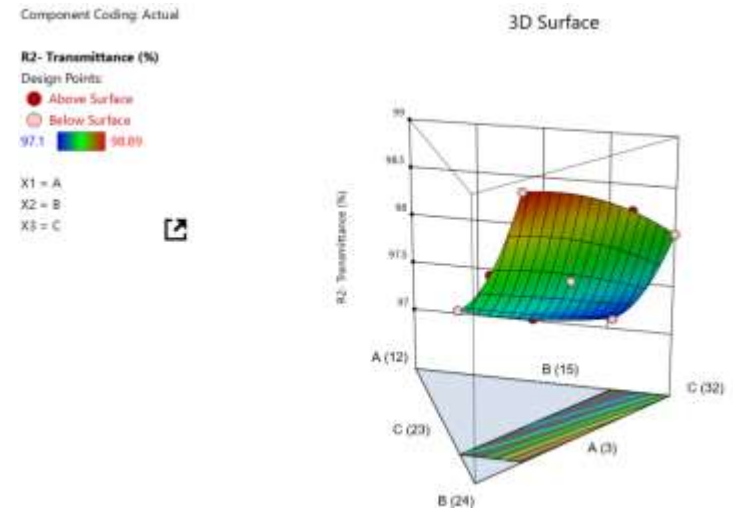


Fig. 10A: Transmittance (%)

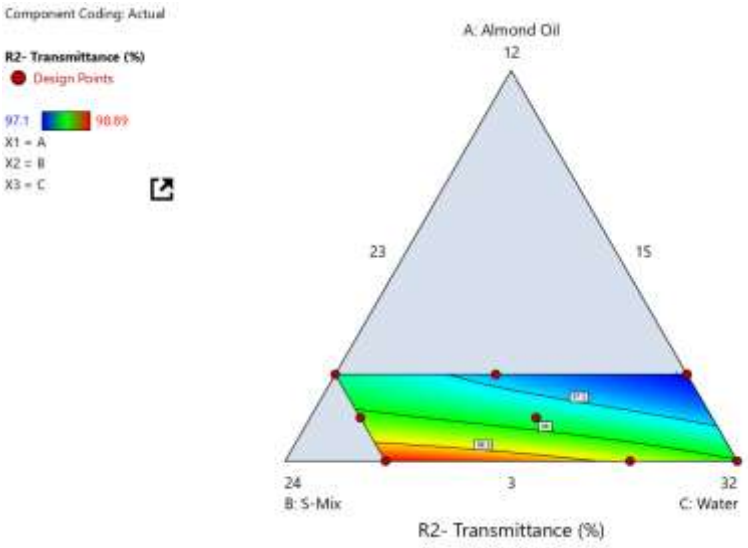


Fig. 10B: Transmittance (%)

R2 Interpretation: The Model F-value of 3125.18 implies the model is significant. The Lack of Fit F-value of 0.49 implies not significant.

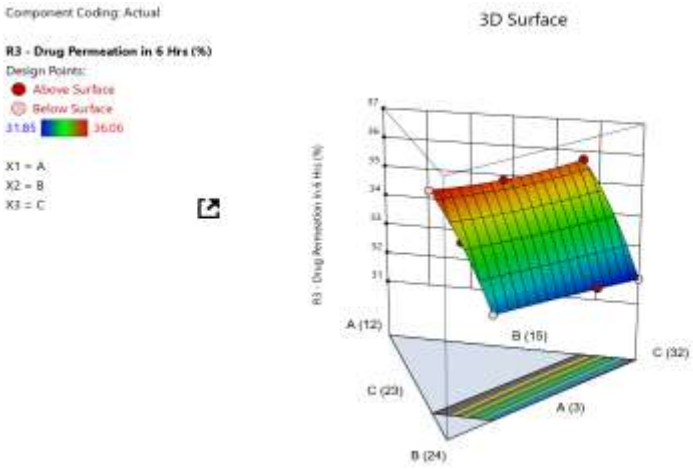


Fig. 11A: Drug Permeation in 6hrs. (%)

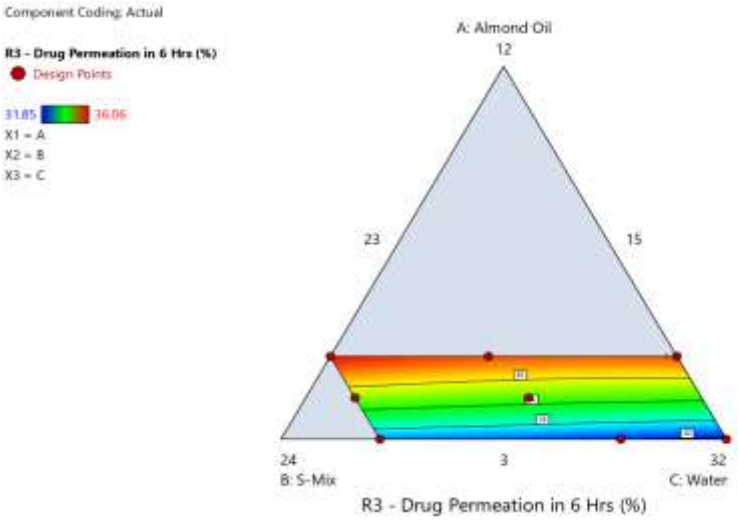


Fig. 11B: Drug Permeation in 6hrs. (%)

R3 Interpretation: The Model F-value of 6423.84 implies the model is significant. The Lack of Fit F-value of 0.56 implies not significant.

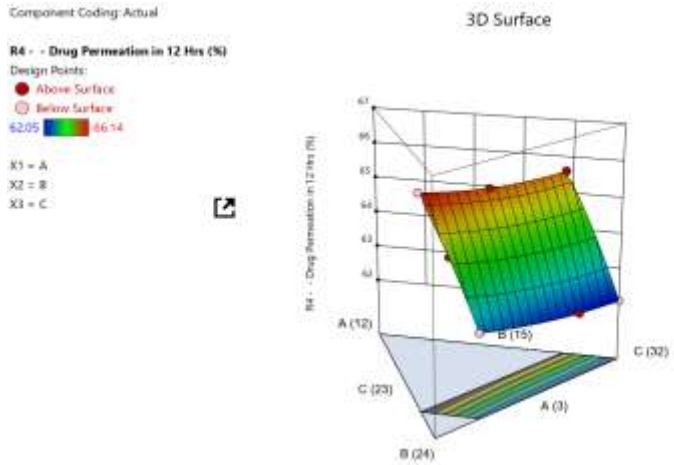


Fig. 12A: Drug Permeation in 12hrs. (%)

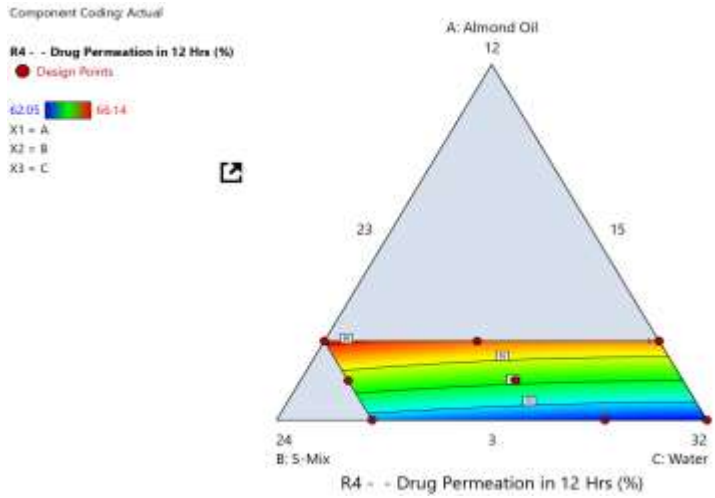


Fig. 12B: Drug Permeation in 12hrs. (%)

R4 Interpretation: The Model F-value of 34884.77 implies the model is significant. The Lack of Fit F-value of 0.02 implies not significant.

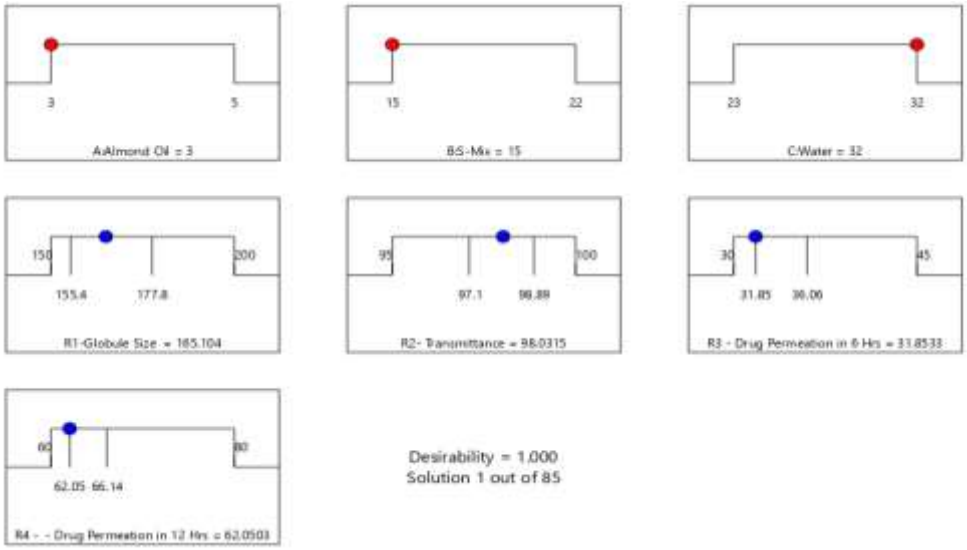


Fig. 13: Optimization Desirability Graph

Table 06: Coefficient Table

	A	B	C	AB	AC	BC	ABC
R1-Globule Size	1192.4	175.679	165.104	**-1322.46**	**-1254.5**	6.38964	-125.517
p-values				**0.0061**	**0.0064**	0.6297	0.2292
R2- Transmittance	104.294	98.9442	98.0315	**-13.7729**	**-13.3843**	**0.87506**	**-5.46992**
p-values				**0.0048**	**0.0048**	**0.0140**	**0.0149**
R3 - Drug Permeation in 6 Hrs	27.0051	32.5476	31.8533	**27.4783**	**28.6022**	0.129505	-4.83018
p-values				**0.0050**	**0.0044**	0.6057	0.0729
R4 - - Drug Permeation in 12 Hrs	66.7442	63.0031	62.0503	**13.3422**	**14.5021**	**-0.75677**	-1.2171
p-values				**0.0035**	**0.0028**	**0.0128**	0.1614

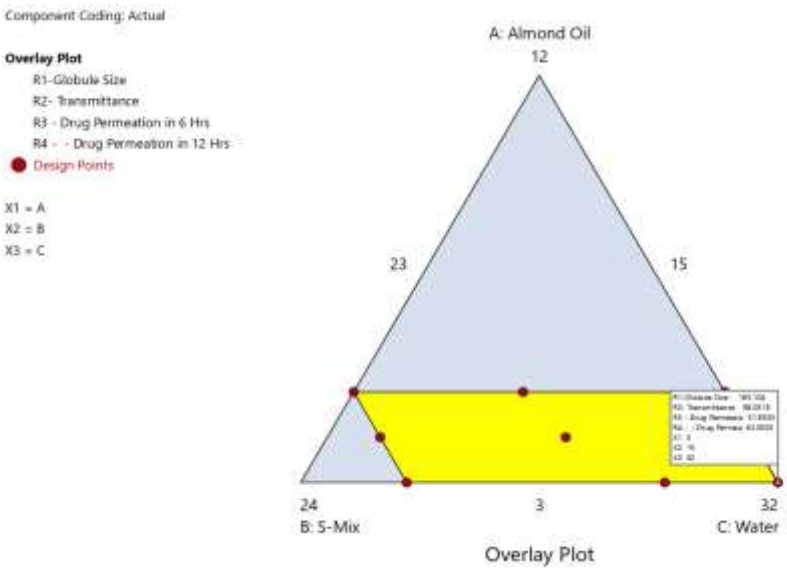


Fig. 14: Overlay Plot

Table 07: Model Comparison: Summary statistics for paroxetine microemulsions responses of R1-Globule Size, R2-Transmittance, R3 - Drug Permeation 6 Hrs. and R4 - Drug Permeation in 12 Hrs.

R1-Globule Size					
Source	**Std. Dev**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**
Special Cubic	0.8743	0.9953	0.9811	0.5015	161.23
Linear	4.37	0.6459	0.5279	0.2902	229.56
Quadratic	1.67	0.9742	0.9311	0.5284	152.50
Cubic	0.0212	1.0	1.0	NA	NA

R2- Transmittance					
Source	**Std. Dev**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**
Special Cubic	0.1079	0.9965	0.9858	0.6267	2.45
Linear	0.1325	0.9840	0.9786	0.9587	0.27
Quadratic	0.0900	0.9963	0.9901	0.9238	0.50
Cubic	0.0071	1.0000	0.9999	NA	NA

R3 - Drug Permeation in 6 Hrs					
Source	**Std. Dev**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**
Special Cubic	0.1590	0.9991	0.9965	0.9082	5.31
Linear	0.6529	0.9558	0.9411	0.9010	5.74
Quadratic	0.3037	0.9952	0.9873	0.9382	3.58
Cubic	0.0141	1.0000	1.0000	NA	NA

R4 - Drug Permeation in 12 Hrs					
Source	**Std. Dev**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**
Special Cubic	0.1977	0.9982	0.9930	0.8153	8.20
Linear	0.4185	0.9763	0.9684	0.9473	2.34
Quadratic	0.3769	0.9904	0.9744	0.8759	5.51
Cubic	0.0212	1.0000	0.9999	NA	NA

4.9 Factorial Design for Ionotophoretic effect

Table 08: 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 09: 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	36.14	360	97.45

2	1	3	0	70.05	240	97.22
3	7	0	3	36.16	360	97.48
4	3	3	3	68.25	200	97.25
5	5	1.5	1.5	71.18	220	97.4
6	4	1.5	1.5	71.25	210	97.41
7	6	1.5	1.5	71.21	220	97.38

Table 10: ANOVA for selected Factorial Model

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio	F-value	p-value
R1	Cmax	%	7.00	36.14	71.25	60.61	16.74	1.97	2.948E+05	< 0.0001
R2	Tmax	Min	7.00	200	360	258.57	70.34	1.80	64.29	0.0034
R3	Drug Content	%	7.00	97.22	97.48	97.37	0.0983	1.00	76.86	0.0129

Interpretation:
Response R1: The Model F-value of 294762.22 implies the model is significant. Response R2: The Model F-value of 64.29 implies the model is significant. Response R3: The Model F-value of 76.86 implies the model is significant. P< 0.05 indicates significant.

4.10 Optimization of 3D Surface

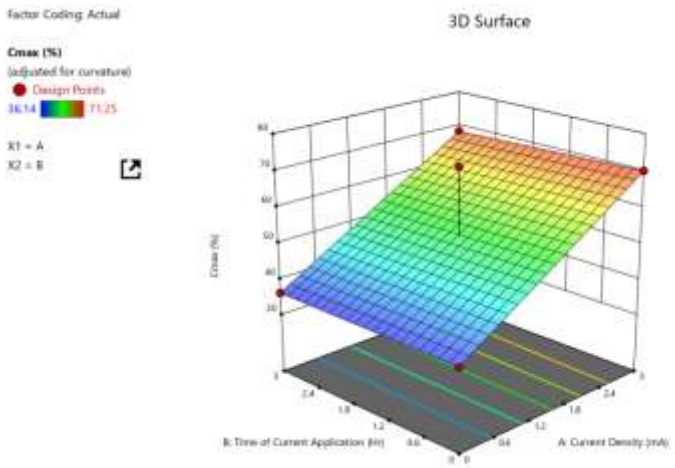


Fig. 15: Cmax (%)

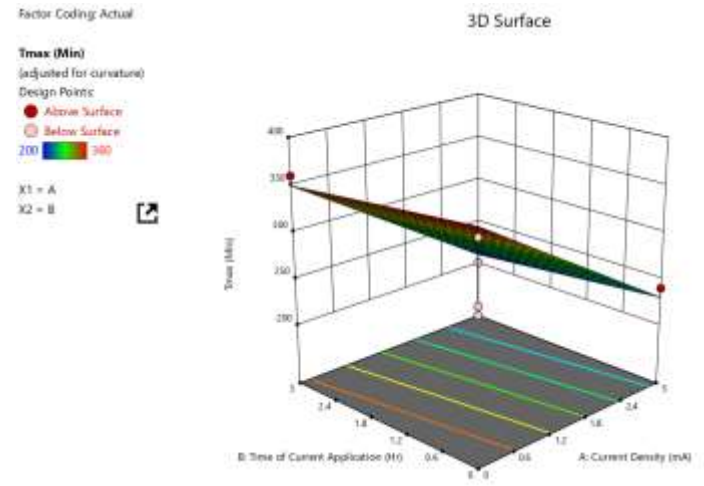


Fig. 16: Tmax (Min)

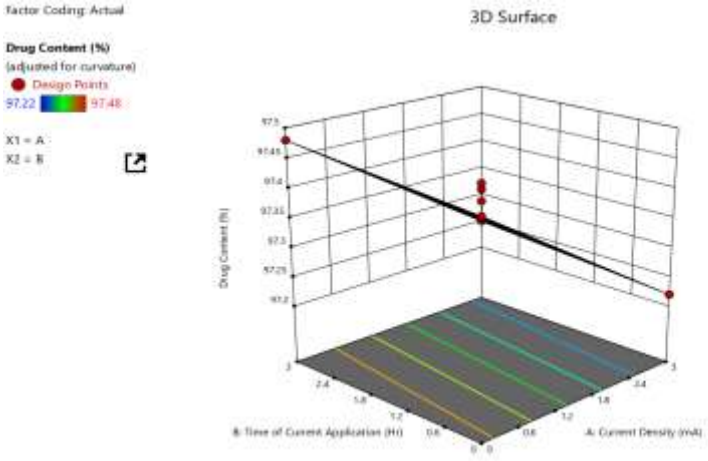


Fig. 17: Drug Content (%)

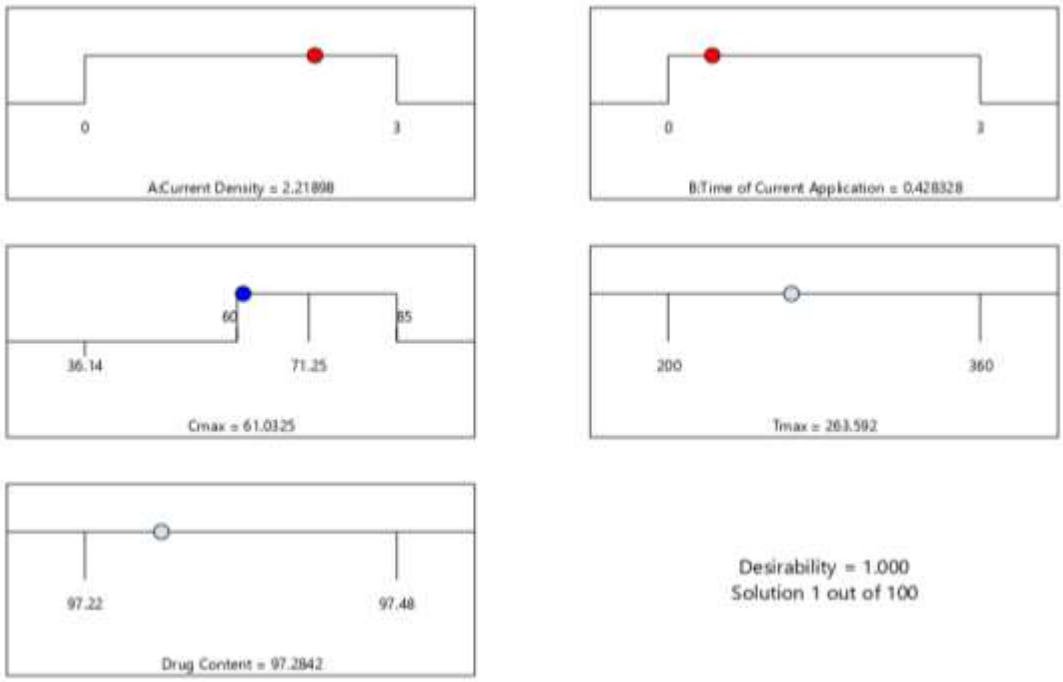


Fig. 18 Optimization Desirability Graph

Table 11: Coefficient Table

	Intercept	A	B	AB
Cmax	52.65	**16.5**	**-0.445**	**-0.455**
p-values		**< 0.0001**	**0.0016**	**0.0015**
Tmax	290	**-70**	-10	
p-values		**0.0015**	0.2071	
Drug Content	97.35	**-0.115**	0.015	4.22793E-15
p-values		**0.0044**	0.1885	1.0000

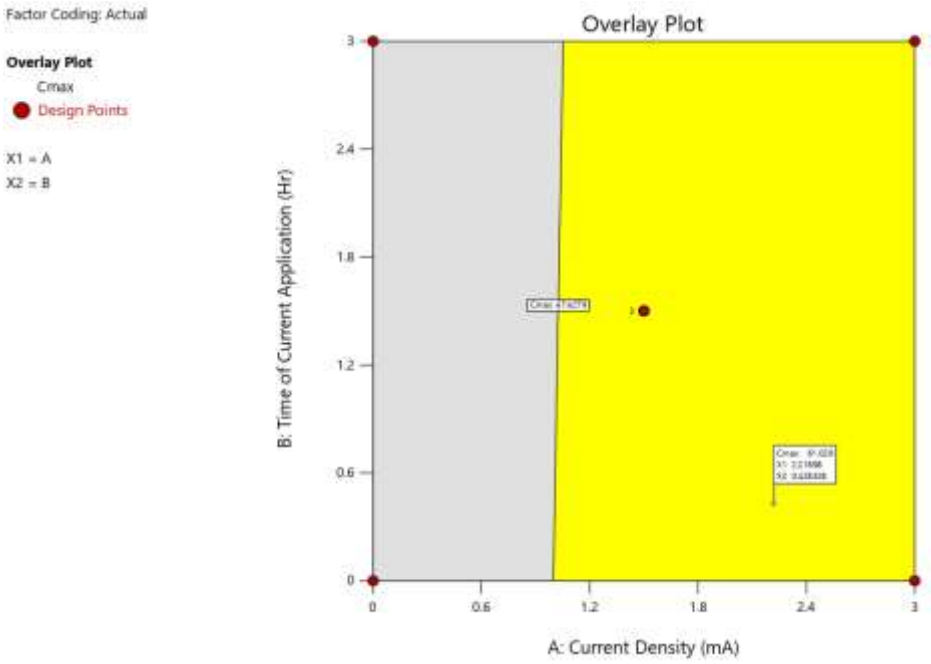


Fig. 19 Overlay Plot Graph

4.11 Observation of Microemulsion by Optical Microscope

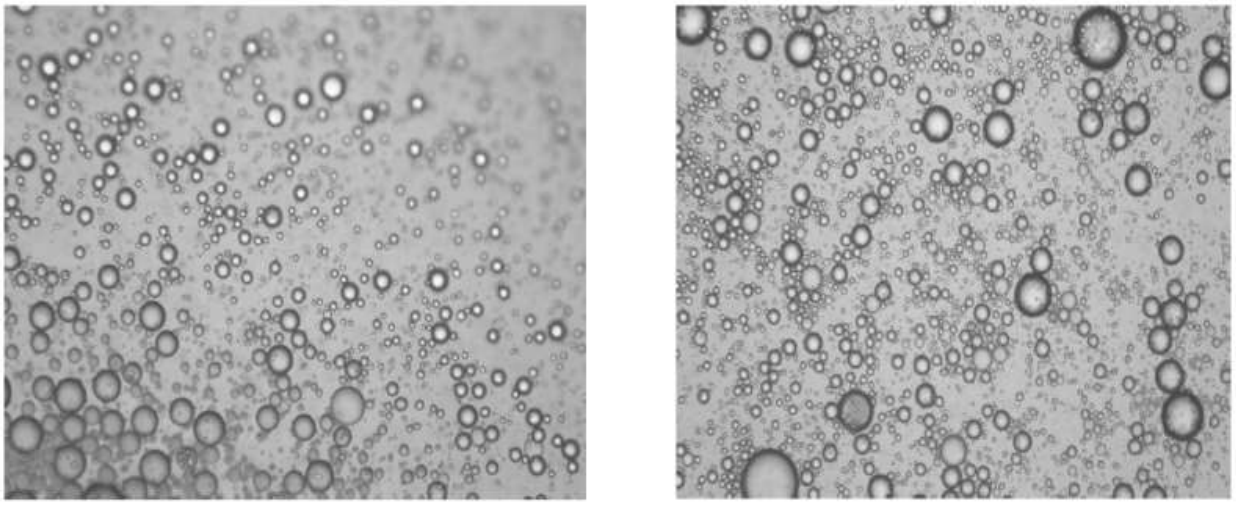


Fig. 20: Optical microscope of globule size of microemulsion of almond oil

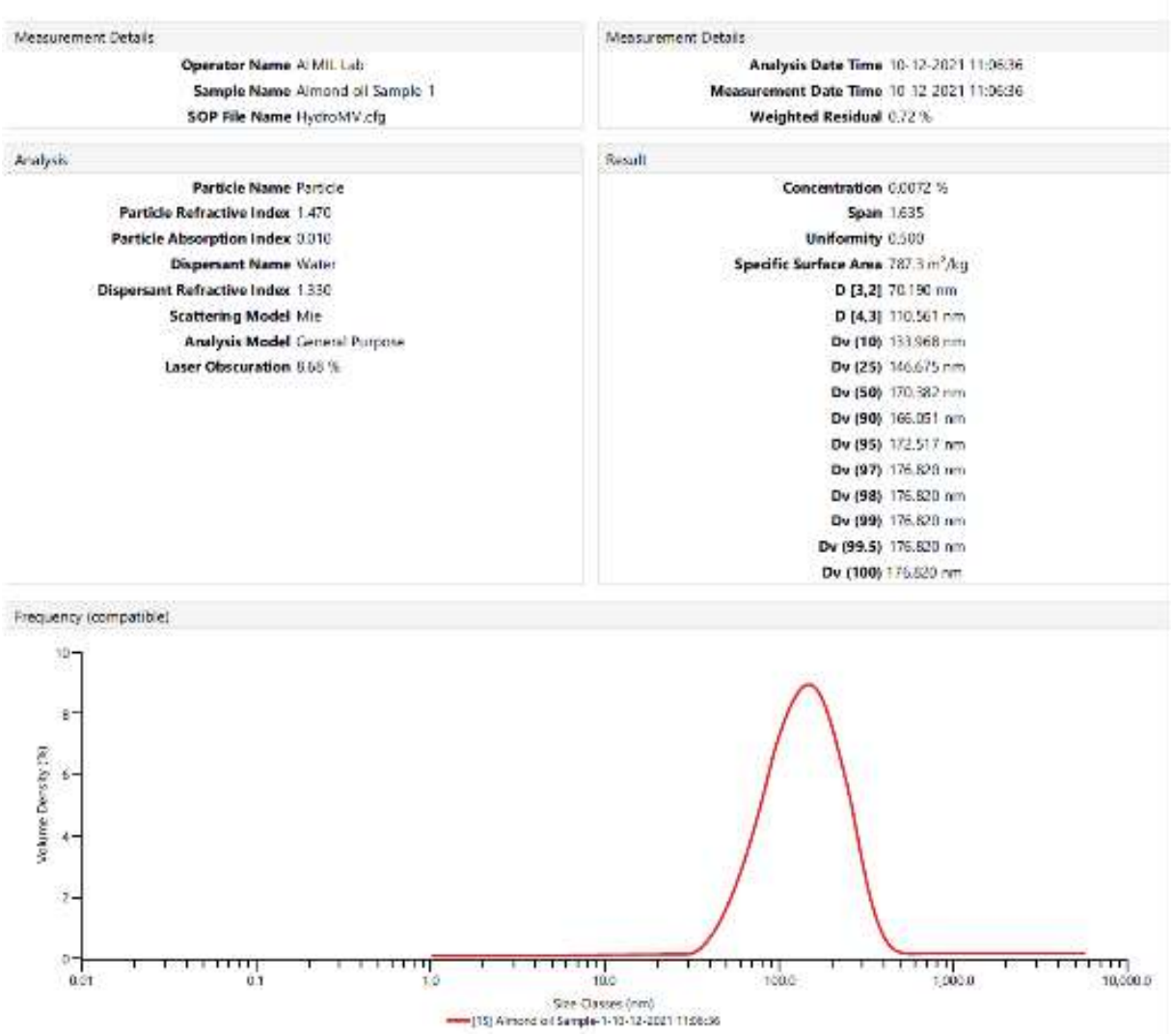


Fig. 21: Particle size distribution of Paroxetine HCl based almond oil microemulsion

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. 21).

Sample Details

Sample Name:	Almond oil Sample-1. 3		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	Karnataka College of Pharm...	**Dispersant Name:**	Water
Record Number:	234	**Dispersant RI:**	1.330
Date and Time:	10 December 2021 11:10:58	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	177.8	**Measurement Position (mm):**	2.00
Cell Description:	Clear disposable zeta cell	**Attenuator:**	6

Results

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-18.86	**Peak 1:**	-18.86	100.0	2.95
Zeta Deviation (mV):	2.95	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0551	**Peak 3:**	0.00	0.0	0.00
Result quality	Good				

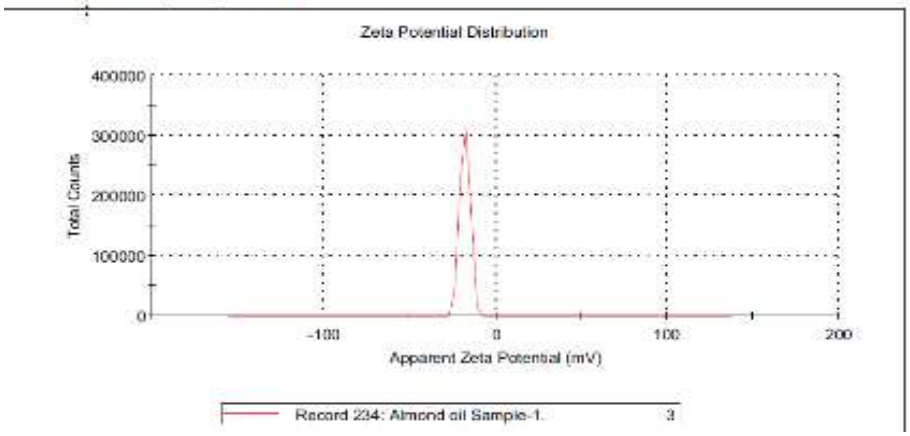


Fig. 22: Determination of Zeta potential

4.12 Differential scanning Colorimetry (DSC)

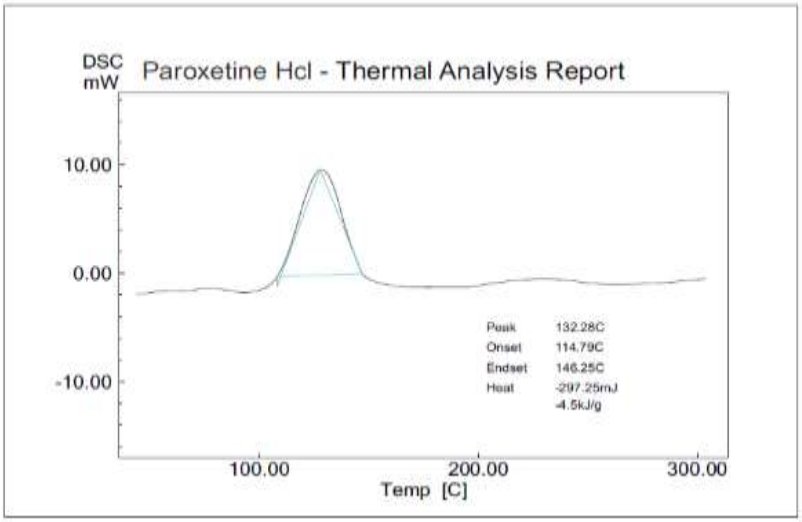


Fig. 23: Paroxetine HCl –Thermal analysis report

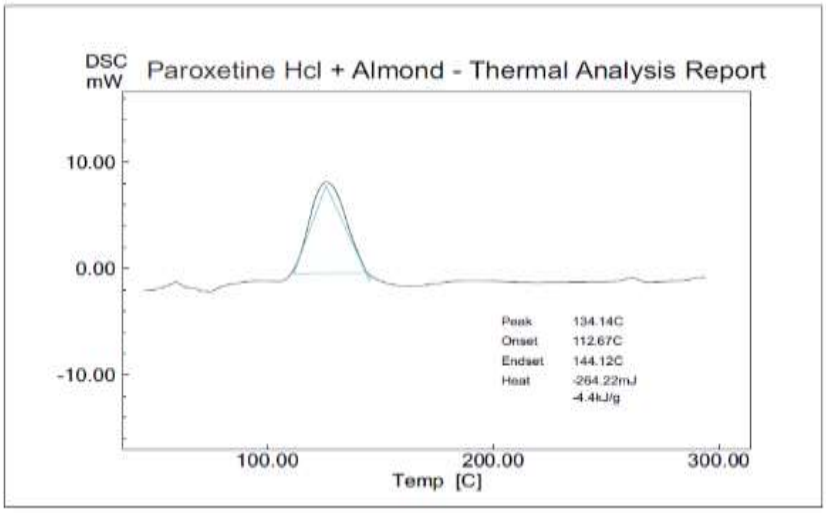


Fig.24: Paroxetine HCl Almond Oil Microemulsion–Thermal analysis report

4.13 In-Vitro Diffusion Study of Optimised Formula

Table 12: 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	11.48082	1.947037382	1	0	1.0599729
2	26.97552952	1.863468416	1.414213562	0.30103	1.43097
3	40.23091524	1.776476606	1.732050808	0.47712125	1.6045599
4	58.43389619	1.618739318	2	0.60205999	1.7666648
5	67.49067238	1.512007987	2.236067977	0.69897	1.8292438
6	74.12112	1.412945477	2.449489743	0.77815125	1.869942
7	81.03192	1.278023373	2.645751311	0.84509804	1.9086561
8	89.12313259	1.036503835	2.828427125	0.90308999	1.9499904

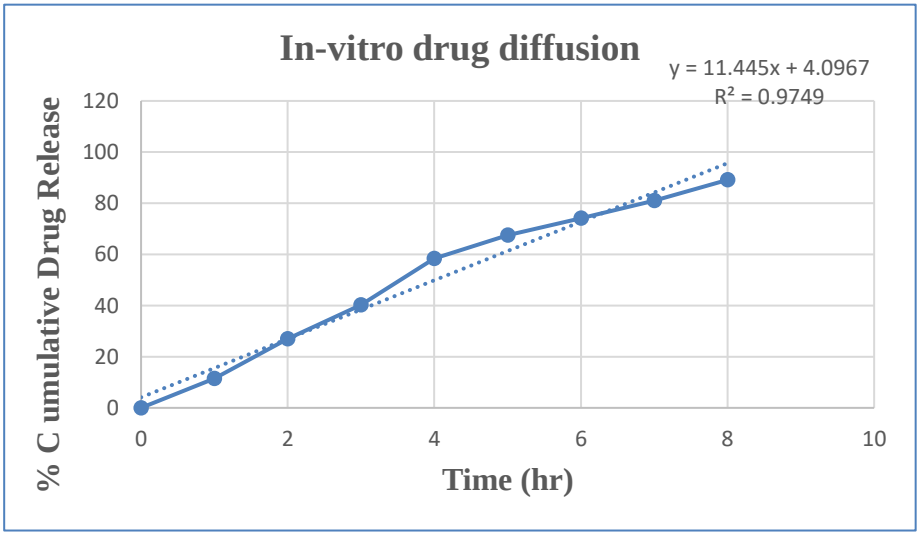


Fig. 25: In-Vitro plot of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

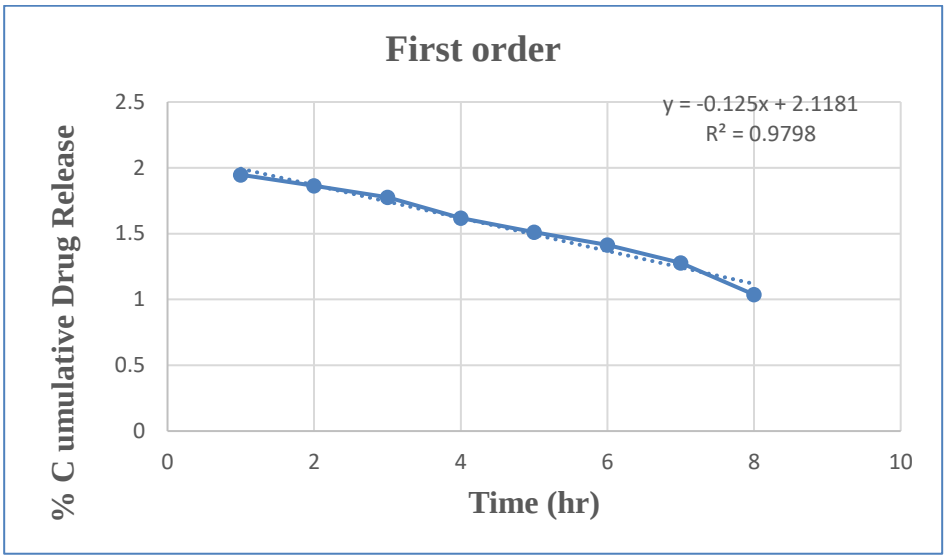


Fig. 26: First order plot of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

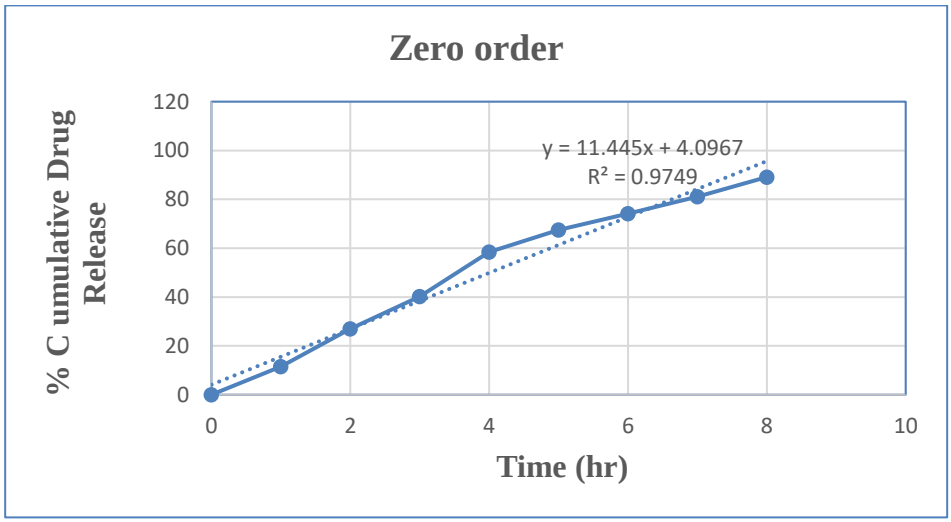


Fig. 27: Zero order of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

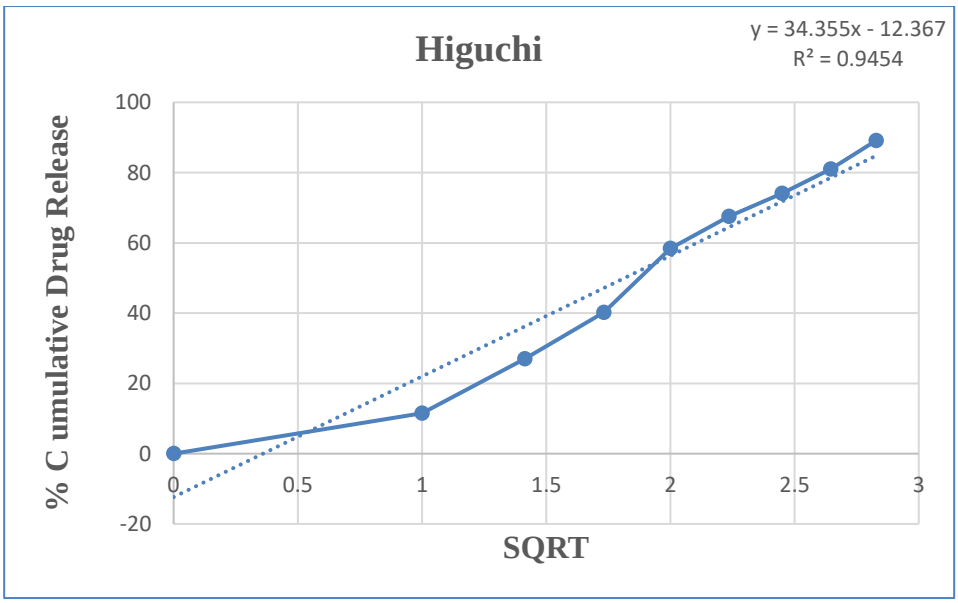


Fig. 28: Higuchi plot of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

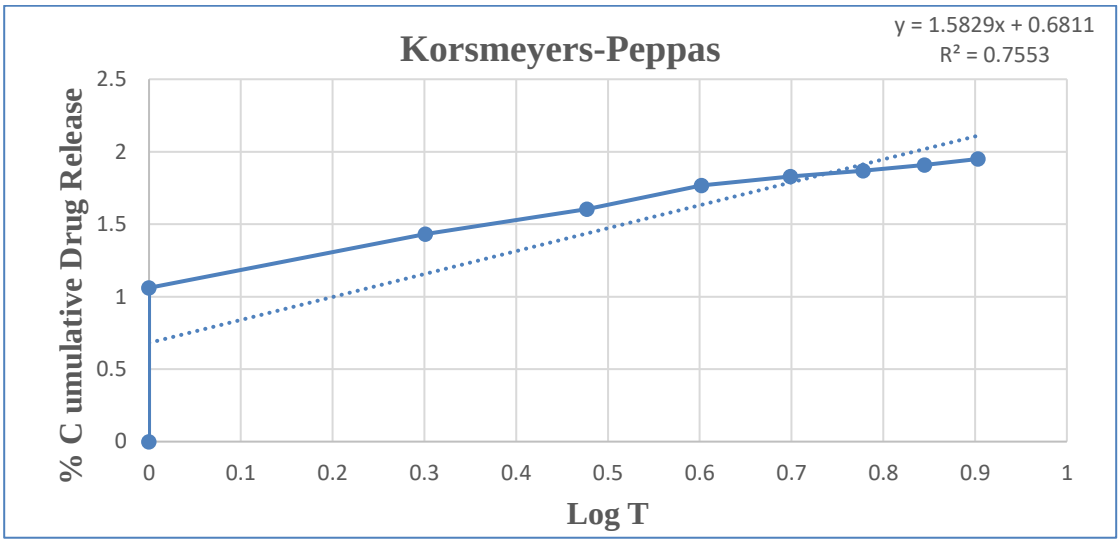


Fig. 29: Korsemeyer-peppas equation of optimized formula of Paroxetine HCl Microemulsion transdermal gel.

4.14 Ex-Vivo Transdermal Permeation Study

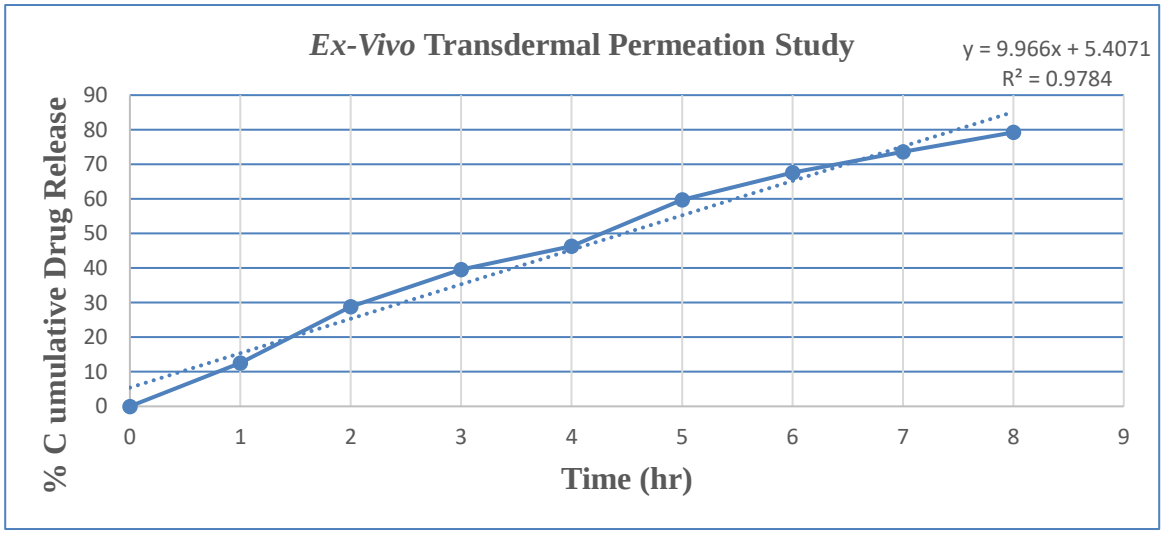


Figure 30: Ex-Vivo Transdermal Permeation Study of Optimized formula

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

Table 13: Stability studies of Paroxetine HCl based Microemulsion Transdermal gel

Physicochemical Properties of Optimised formulation		Days		
		30	60	90
% Transmittance	A	98.89 ± 1.85	98.45 ± 1.73	97.12 ± 1.44
% Cmax	A	71.24 ± 2.58	71.12 ± 1.54	69.54 ± 1.64
Tmax (min)	A	360 ± 2.54	345 ± 3.75	324 ± 4.56
% Drug Content Uniformity	A	97.48 ± 1.57	97.54 ± 1.49	97.29 ± 1.26
% Cumulative drug diffusion	A	85.12 ± 1.29	79.29 ± 1.53	74.12 ± 1.67
% Cumulative drug Release	A	79.23 ± 2.44	73.64 ± 2.37	67.58 ± 2.23

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.[19] Approximately 40% concerning new chemical entities shows less soluble in H_2O and provides a tremendous 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.[20] 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 was 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. The λmax was found to be a 271 nm which is shown in fig. 02. Preformulation studies have been carried out by adding the drug with different excipients in various proportions, and the result was no significant changes appear in the sample. FT-IR interpretation were done and shown there was no incompatibility between drug and excipients (fig. 06-08). Solubility studies were obtained in different oils, surfactants and co-surfactants and which shown more soluble were selected for the preparation of microemulsion gel (fig. 04). 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 09 and 10). 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 have been subjected to different thermodynamic stability 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 also checked by transparency, measured in terms of % T. The % transmittance was found in the range of 97.1% - 98.89%. 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 greater absorption over microemulsion may keep attributed in imitation of nano sized fine droplets as observed of droplet size analysis. This increased surface area has direct affect over the improved contact with intestinal mucosa and therefore higher absorption. This is definitely indicated of reduced Tmax for the microemulsion (Fig. 20). 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. 21 and 22). DSC was found to be in fairly acceptable (Fig. 23 and 24). The % Drug content of optimized formulation was found to be 97.37%, which complies with pharmacopeial specifications (Table 09 and 10). *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.12. The *In-vitro* drug diffusion of optimized formulation was found to be 89.12% 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. 25 to 29). 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 79.23% at 8hr (Fig. 30). Accelerated stability studies were performed as per the ICH guideline over a period of 3 months. The results of the studies are tabulated in Table 13 shown that the microemulsion did not change significantly in their physical appearance, assay and % CDR. Therefore we can conclude that the microemulsion formulation is stable.

CONCLUSION

The current study hold a satisfactory strives to formulate a Paroxetine HCl microemulsion for transdermal gel. From the experiments, it may be concluded that, microemulsion of Paroxetine HCL have been prepared by using Almond Oil (3ml), surfactant mixture consisted of Tween®80 & Propylene glycol in 1:3 ratio (15ml) and DDW (32ml). The FTIR result was the indication of no interaction between drug and excipients. PDI and Zeta potential were observed and the mean particle size and microparticles distribution was in the range, % Transmittance, Cmax, Tmax, drug content and % CDR have been found to acceptable range. Optical microscope was observed global size of microemulsion studies indicate surface topography having round surface of the molecules, DSC was recorded to see the drug status. *In-vitro* and *ex-vivo* have been shown a significant impact on drug release. Stability studies have revealed that optimized formulation was fairly stable. Finally it was concluded that the formulation Almond oil microemulsion of Paroxetine HCl transdermal gel may prove to be a capable enough to achieve for effective drug delivery.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS

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