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Original Research Paper

FORMULATION AND OPTIMIZATION OF THERMOREVERSIBLE *IN-SITU* GEL OF VENLAFAXINE HCl

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ABSTRACT

The purpose of this research was to study the in-situ nasal gel system containing Venlafaxine HCl suitable for administration in nose was formulated by cold method using combination of Poloxamer 407 and Polyvinyl pyrrolidone (PVP). Thermoreversible, bioadhesive polymers such as Poloxamer 407 and PVP in the form of in situ gel by cold technique. To modulate the gel strength and bioadhesive force for Venlafaxine HCl nasal gel, bioadhesive polymers PVP were investigated. The gels were evaluated for gelation temperature, bioadhesive force, gel strength, viscosity, Drug content and in-vitro release. A 2-factor, 2-level full factorial design (2^2) was employed for optimization of Venlafaxine HCl gels with Poloxamer 407 amount (%, X1) and polymers (PVP %, X2) as the prime selected independent variables, which were varied at 2 different levels (low and high). The effect of formulation variables on the response variables were statistically evaluated by using a commercially available software package Design-Expert® version 8.0.1. The results revealed that as the increase of bioadhesive polymer PVP concentration, decrease in gelation temperature (T). pH of all formulation were found to be within the range between 6 to 6.5. The drug content for all formulation was found to be 96%-100%. The mucoadhesive test indicates that the level of PVP increases, the mucoadhesive strength also increases. The developed formulations had optimum viscosity. The optimized formulation shows the controlled drug release. This study further demonstrates that administration of Venlafaxine Hcl intranasal in gel form is a pleasant and painless alternative.

Keywords: Nasal drug delivery, Venlafaxine HCl, Gel, Poloxamer 407, PVP, Factorial design.

INTRODUCTION

The nasal drug delivery shows many advantages such as nasal drug delivery avoid hepatic first-pass elimination, it shows rapid absorption/fast onset of action due that therapeutic window achieve rapidly by drug, nasal drug delivery system also possible direct pathways to the CNS bypassing the blood–brain barrier, olfactory region is direct pathways to CNS due to that pathways we can targeted CNS and treat various CNS related diseases.[1,2] The nasal drug delivery system having some disadvantages like in nasal drug delivery system we can administered only limited to potent drugs/small volumes (25–200 µl), another disadvantage is active mucociliary clearance due to that retention time of drug get reduces, large molecules shows poor penetration across the nasal mucosa, Delivery is expected to decrease with increasing molecular weight of drug and nasal congestion due to cold or allergies may interfere with nasal drug delivery system.[2,3] Very good results were obtained with small organic molecules, which led to the successful development of a number of products currently on the market, list of products that is steadily increasing. Examination of the causes of failure led to the conclusion that the short residence time of the formulation within the nasal cavity coupled to the low permeability of the latter did play

significant roles.[4,5] Consequently, the attention shifted to the evaluation of mucoadhesive polymers, some of which would even demonstrate additional permeation- enhancing capabilities.[6] The encouraging results and the desire to overcome some new challenges stimulated the development of new generations of polymers based on pH or thermal responsiveness or modified existing polymers having improved bioadhesive or permeation-enhancing properties.[6] In last decade, much interest has been given to the exploitation of the nasal route for delivery of drug to the brain via specific side, the olfactory region. When we use nasal route for treatment of CNS disorder it bypass BBB that's why we can deliver hydrophilic and in some cases high molecular weight drug also.[7] Hence it was decided to develop a formulation of Venlafaxine Hydrochloride thermoreversible gel and optimization done by experimental factorial design, evaluate its various evaluation parameter like drug release profile, mucoadhesion force, gelation time etc.

MATERIALS AND METHODS

Materials

Venlafaxine Hydrochloride was kindly gifted by Cipla (Kurkum) Pune India. Poloxamer 407 was gifted by BASF India Ltd., Mumbai India. PVP, Methyl paraben, Propyl paraben were purchased by Loba Chemical Pvt. Ltd., Mumbai, India.

Methods

Preparation and Optimization of Thermoreversible PF127 Gels

Visual inspection method was used for the optimization of Poloxamer. This method depends upon the visual inspection of gelation temperature. Gelation temperature, defined as the temperature at which the liquid phase makes a transition to gel, was determined as described previously.[8,9] Thermoreversible polymer-based liquid formulations that provide in situ gelling property in nasal cavity were designed to delay clearance of the formulations from the nasal cavity. It was found that gelation temperature decreases as the concentration of Poloxamer increases and sol temperature increases. Below table shows different sol-gel temperature obtained from visual inspection method. In the preliminary studies, the minimum concentration of Poloxamer 407 that formed gel near to the body temperature was found to be 18% wt/vol. In general, the gelation temperatures have been considered to be suitable if they are in the range of 25 ^{0}C to 37 ^{0}C.[9]

Factorial Batches for Poloxamer and PVP

A 2^2 factorial design was implemented for optimization of combination optimum polymeric formulation. According to the model it contained 2 independent variables at 2 levels, +1,-1. According to model total four formulations are possible, the composition of different formulation are shown in Table 8.1 The different independent variables, were Poloxamer concentration % w/w (X_1) and PVP concentration % w/w (X_2). Dependent factors included % drug release at 8 hrs gelation Temperature and mucoadhesive force. Preparation of factorial batches as per table no. 1.[10, 11]

Preparation of Mucoadhesive Thermoreversible Nasal Gels

Venlafaxine Hydrochloride along with mucoadhesive polymers (PVP), Methyl paraben and Propyl paraben were stirred in the calculated amount of distilled water at room temperature. The dispersions were cooled down to 4 ^{0}C in refrigerator; the Poloxamer was added slowly with continuous stirring. The dispersions were then stored in a refrigerator for overnight until clear solutions were obtained. Finally, volume was adjusted with distilled water. All final formulations were evaluated for their clarity, pH, content uniformity, gelation temperature, mucoadhesive strength, viscosity study and diffusion study. Formula mentioned in table no. 2.[10,11]

Evaluation of Formulations

Clarity

The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows; turbid: +, clear: ++, very clear: +++.[11]

pH

The pH of each formulation was measured using pocket pH meter which was calibrated using buffers of pH 4 and pH 7 before the measurements. Each recording was made in triplicates when they are in sol condition.[11]

Drug Content

Tests for drug content were carried out for all the prepared gel formulations. The vials (n=3) containing formulation were properly shaken for 2-3 min. 0.2 ml from each formulation was taken in 100 ml volumetric flask, dissolved in phosphate buffer pH 7.4 with gentle stirring and final volume was adjusted. The volume 1 ml from this solution was diluted up to 100 ml with phosphate buffer pH 7.4 to obtain concentration 2µg/ ml respectively. The absorbance was measured at analytical wavelength 225 nm using phosphate buffer pH 7.4 as blank using Shimadzu 1700 spectrophotometer.[12,13]

Gelation Temperature

The volume 2 ml of prepared Poloxamer solution was added in to test tube. Then these test tubes were placed on the water bath to heat. The temperature was increased in steps of 1^0C/minute.The gelation temperature was measured the gel not follow down when test tube in invert position due to gelation. The temperature was allowed to increase with constant rate until the gel again comes in liquid form to measure sol temperature. Each preparation was tested thrice to control the repeatability of the measurement. Measurement was carried in triplicates for each formulation.[12,13]

Determination of Mucoadhesive Strength

Nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissues were immediately used after separation. This section of nasal tissue instantly fixed with mucosal side out on to glass slide using rubber band. The slide with nasal mucosa was stored at 37 ^{0}C for 5 minutes. Then this glass slide was connected to the right arm of the balance in inverted position. The nasal mucosa was hydrated with distilled water prior to mucoadhesion testing. The slide with nasal mucosa fixed to height adjustable stand. The fixed amount of each gel formulations were placed on the lower slide with nasal mucosa. The lower slide was then elevated till the surface of the sample came in contact with the nasal mucosa. Both the in situ gel and the hydrated nasal mucosa were left in contact for 2 min using a preload of 10 g to establish the contact between them and allow the formation of an adhesive bond. The preload time and force were kept constant for all the tested formulations. The nasal mucosa was detached from the tested sample and the weight required to detach the tested sample from the nasal mucosa was calculated by difference. The results were the mean of three runs. The detachment force (dyne/cm2) was determined using the following equation[14]

Detachment force (dyne/cm^2) = mg /A

Where as,

m: the weight of water in grams;

g :acceleration due to gravity taken as 980 cm/sec^2; and

A: the area of the mucosa (area of contact) and is equal to πr^2 (r is the radius of the mucosa).

Viscosity Study

The viscosities of various formulations were determined by using Cone and Plate viscometer (Brookfield viscometer Model Cap 2000+2). Few drops of formulation were applied to lower plate of the viscometer using glass rod. The temperature was increased in steps of 2 ^{0}C/minute, from 28 ^{0}C to 36 ^{0}C. The apparent viscosity was measured as a function of the temperature (^{0}C).[14,15]

Differential Scanning Calorimetry (DSC) Study

The DSC study was carried out for Venlafaxine Hydrochloride. The DSC patterns were recorded on a METTLER TOLEDO DSC1. Thermographs were obtained by heating 1-5 mg samples in crimped aluminum pans at heating rate of 10 ^{0}C/min, from 30 ^{0}C to 300 ^{0}C, in a nitrogen atmosphere (flow rate 40 mL/min). Data was analyzed, using STAR^e SW 9.20 software, for origin to obtain onset temperature (Tonset); the peak temperature (Tpeak); and the endset temperature (Tendset) of endothermic peak. To evaluate polymorphic properties in physical study

of drug in selected formulations and compared with pure drug.[11,15]

Infrared Spectroscopy study

Fourier transform – infrared (FT-IR) spectra of each formulation were obtained by using FT-IR Spectrophotometer (Brukr). The spectra were scanned over wavelength range of 4000 to 400 cm^{-1} at resolution of 4 cm^{-1}. The KBr pellet techniques were used for the infra-red absorption. The procedure consists of dispersing samples in KBr and compressing into discs by applying a pressure of 5 tons for 5 minutes in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained.[15,16]

Ex-Vivo Diffusion Study

Ex- vivo study carried for all batches. Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. The mucosa was stored in normal saline after removal of blood and bony cartilage from mucosal membrane it becomes ready to use. Tissue samples were inserted in nasal diffusion cells displaying a permeation area of 3.14 cm2. 60 mL of phosphate buffer solution pH 7.4 at 34°C was added to the acceptor chamber. The temperature within the chambers was maintained at 34±1°C. Formulations equivalent to 20 mg of Venlafaxine Hydrochloride were placed in the donor chamber. At predetermined time interval (30 min, 1hr, 2 hr up to 8 hr), 0.5 mL samples were withdrawn from the receptor compartment, replacing the sampled volume with Phosphate buffer solution pH 7.4 after each sampling, for a period of 8 hours. The samples withdrawn were filtered and analyzed spectrophotometrically. The amount of permeated drug was determined using a UV Vis. Spectrophotometer at 225 nm (Linearity range = 1 µg/mL to 10 µg/mL, R^2 =0.997). This procedure was done in triplicate manner for each formulation.[16]

Data Analysis of Release kinetics

To analyze the mechanism for the release and release kinetics of the dosage form, the data obtained was fitted in to Zero order, First order, Higuchi matrix, and Peppas model. By comparing the R^2 values obtained, the best-fit model was selected for developed formulation.[17,18]

Stability Study

The 42 days stability studies were carried out on optimized formulation according to International Conference on Harmonization (ICH) guidelines. Two batches A_2 and B_1 were selected for stability study. Gel was tested for stability under the actual conditions of storage (room condition and accelerated temperature (40 ± 0.5°C relative humidity of 75%). Sufficient quantity of formulation in vials was stored in stability chamber which gives 40^0C temperature and relative humidity of 75% and samples were withdrawn at 0,7,14, 21, 28, 35 and 42 days and evaluated for drug content, gelation temperature and pH.[6, 16,19]

Regression Analysis

The effect of formulation variables on the response variables were statistically evaluated by applying one way ANOVA at $P< 0.05$ level using a commercially available software package Design-Expert® version 8.0.1.6 (Stat-Ease Inc.). To describe the response surface curvature, the design was evaluated by quadratic model, which bears the form of equation and result are reported in result.[20]

RESULTS

Preparation of Batches Factorial Design:

Table 1: Factorial design for preparation of batches

Batches Code	Variable Level In Coded Form	
	X_1	**X_2**
A_1	-1	-1
A_2	+1	-1
A_3	-1	+1
A_4	+1	+1

Table 2: Translation of Coded Value in Actual Unit

Variable level	Low (-)	High (+)
X1= Poloxamer concentration % w/w	18 %	20 %
X2= PVP concentration % w/w	0.3 %	0.5 %

Table 3: Thermoreversible Poloxamer Gel Composition

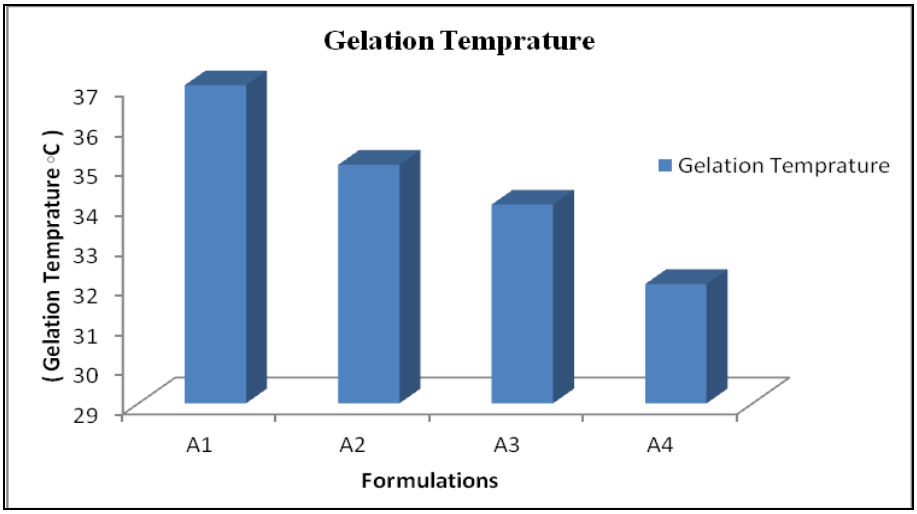
Formulation Code	Poloxamer 407	PVP	Venlafaxine Hydrochloride	MP : PP	Dist. Water
A1	18	0.3	20	0.18:0.02	Q.S.
A2	18	0.5	20	0.18:0.02	Q.S.
A3	20	0.3	20	0.18:0.02	Q.S.
A4	20	0.5	20	0.18:0.02	Q.S.

All concentration in % w/v, MP: Methylparaben, PP: Propylparaben.

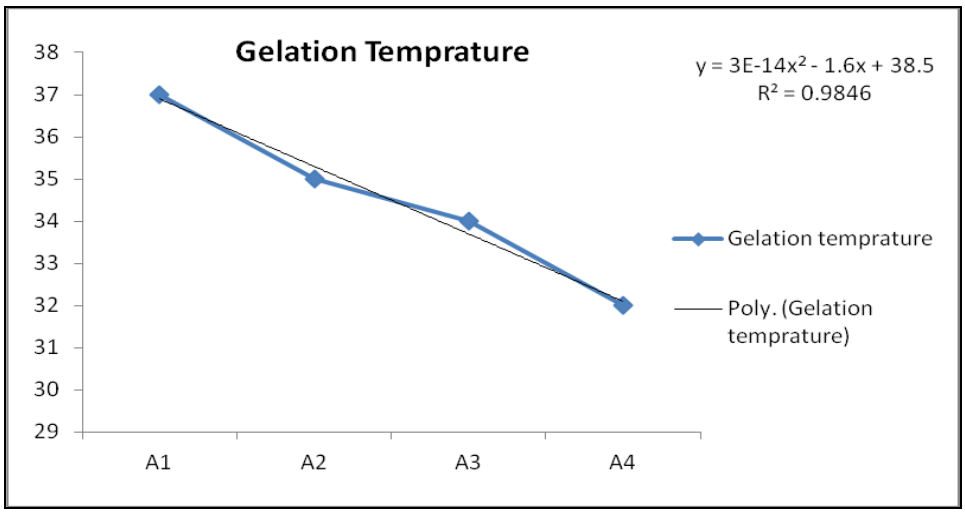
Table 4: Clarity, pH, Gelation temperature, Drug content and Mucoadhesive strength of formulations

Batch Code	Clarity	pH n=3	Gelation Temp. (^{0}C) n=3	Mucoadhesive Strength (dyne /cm^2)	Drug content (% w/v) n=3
A_1	+++	6.8	37	6697.18	98.35±0.58
A_2	+++	6.8	35	7997.61	98.46±0.57
A_3	+++	6.9	34	9167.99	98.91±1.15
A_4	++	7	32	10175.82	101.89±1.32

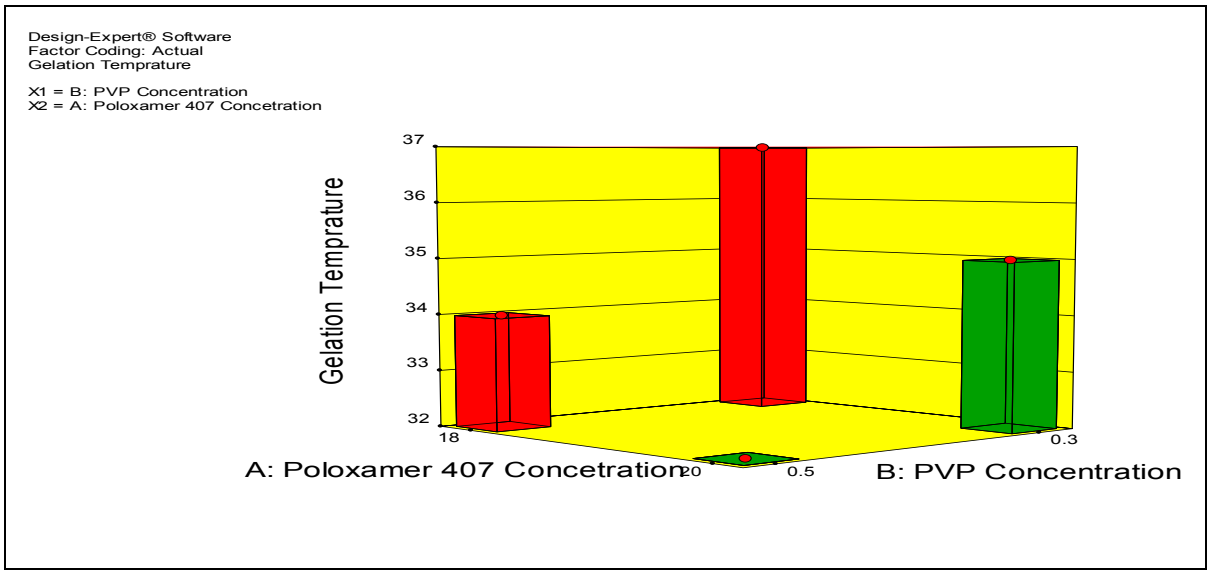
Gelation temperature of formulations:



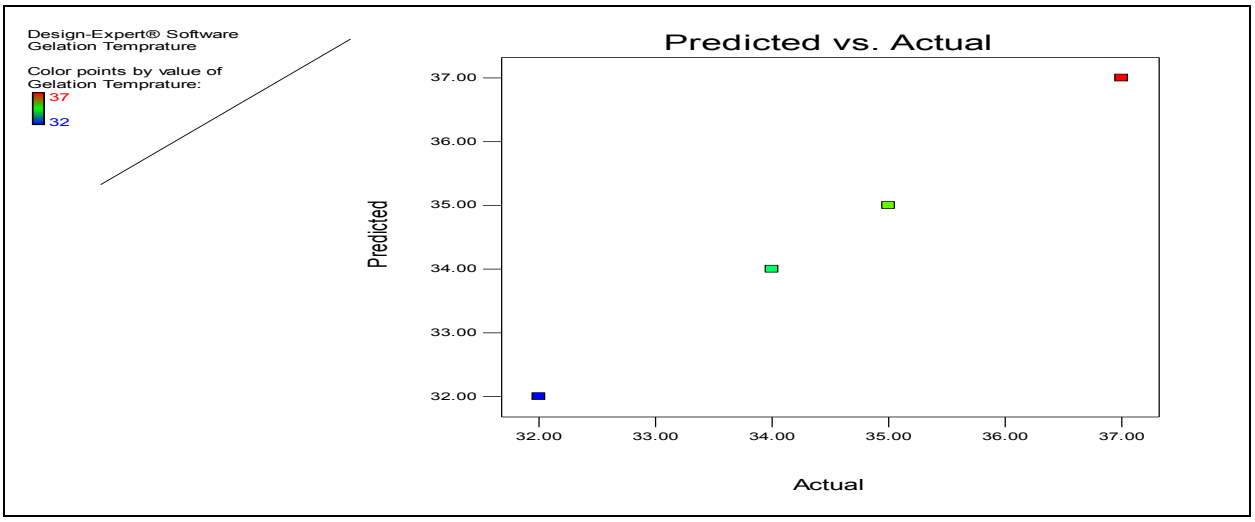
Graph 1: Gelation Temperature



Graph 2: Polynomial equation for Gelation Temperature

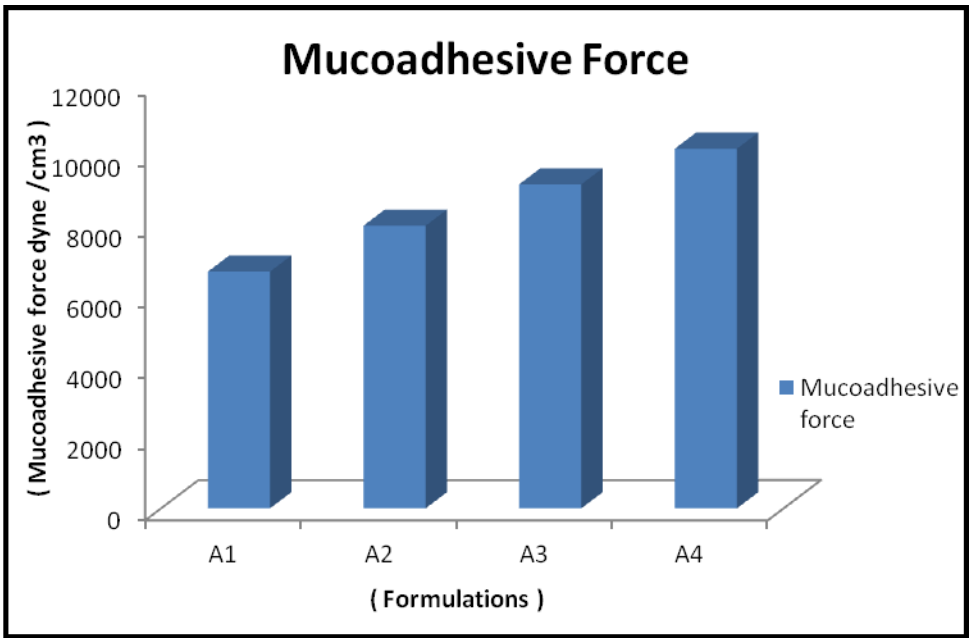


Graph 3: Effect of main factor on Gelation Temperature presented by 3D surface plot of Poloxamer 407: Polyvinylpyrrolidone



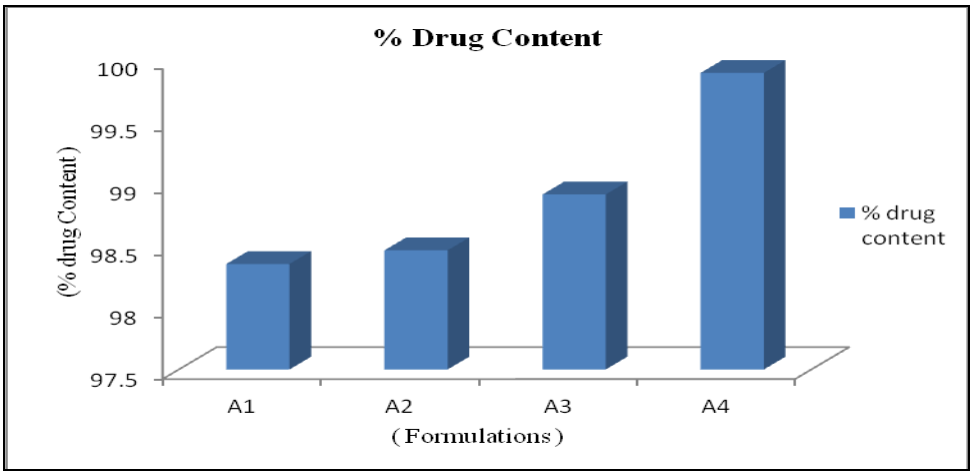
Graph 4: Effect of main factor on Gelation Temperature presented by linear plot of Poloxamer 407: Polyvinylpyrrolidone

Mucoadhesive strength of formulations

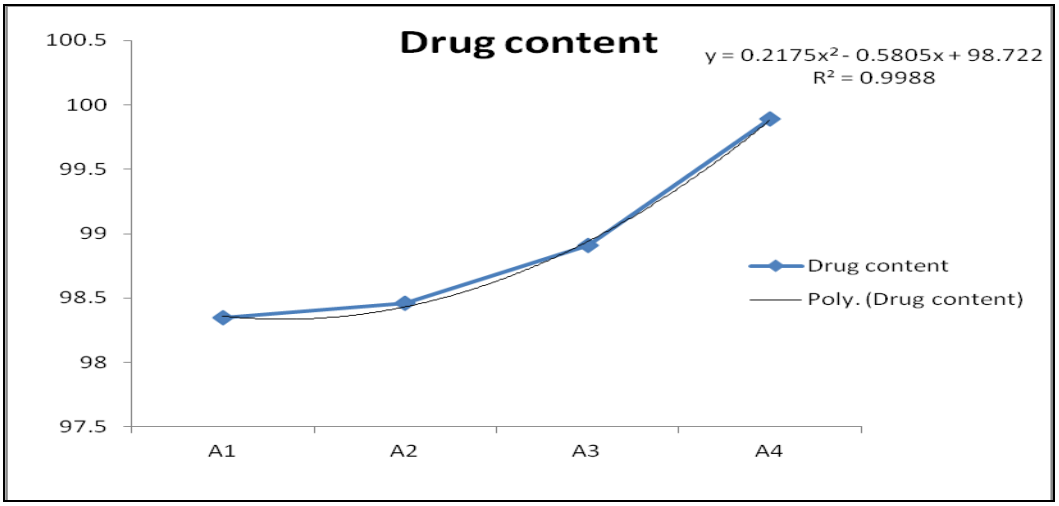


Graph 5: Mucoadhesive Force

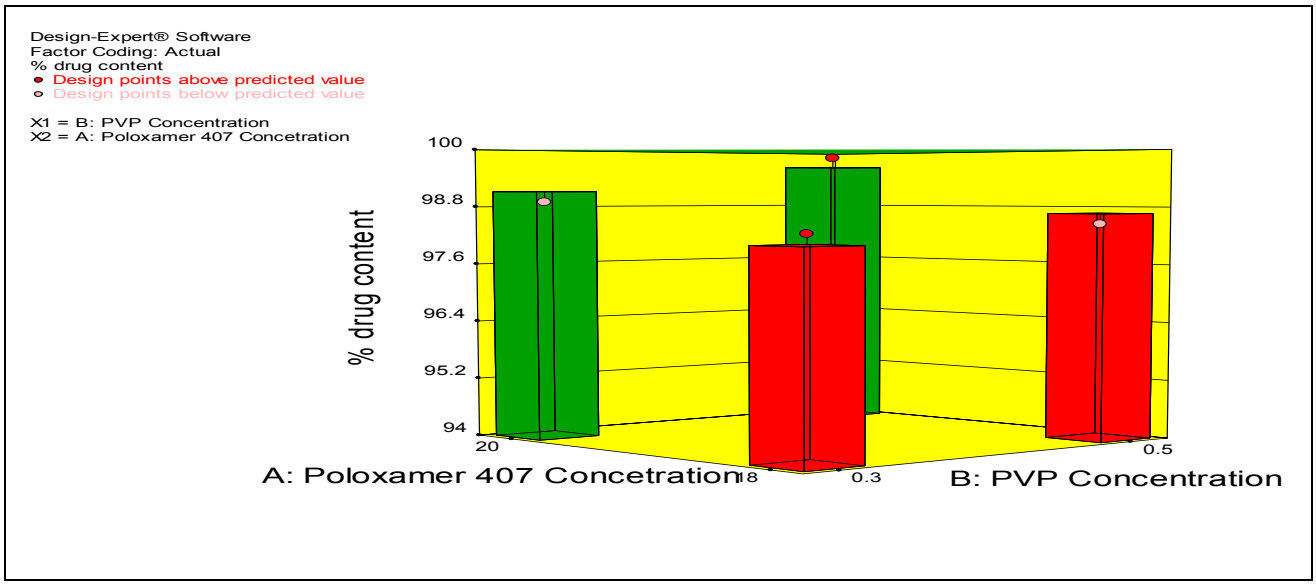
Drug content of formulations



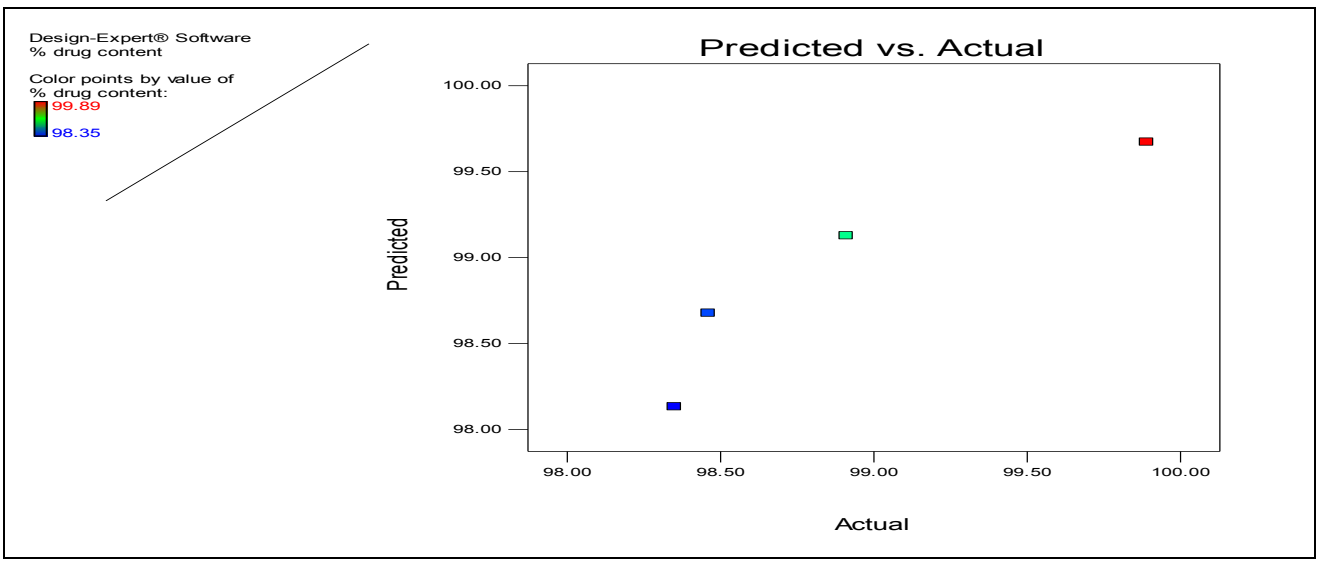
Graph 6,7: % Drug Content



Graph 8: Polynomial equation for % Drug Content



Graph 9: Effect of main factor on % Drug Content presented by 3D surface plot of Poloxamer 407: Polyvinylpyrrolidone

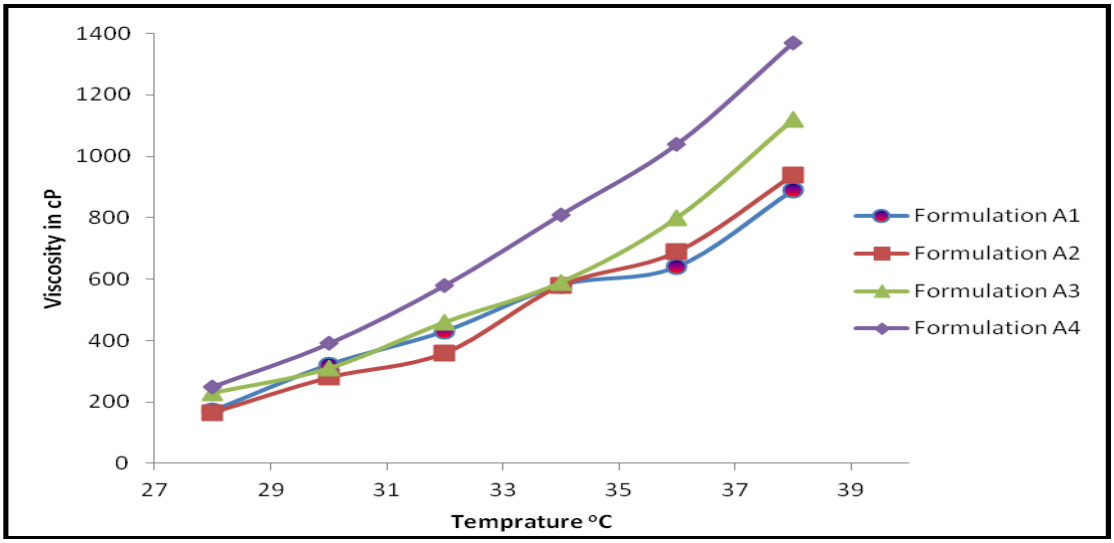


Graph 10: Effect of main factor on % Drug Content presented by linear plot of Poloxamer 407: Polyvinylpyrrolidone

Viscosity of formulation

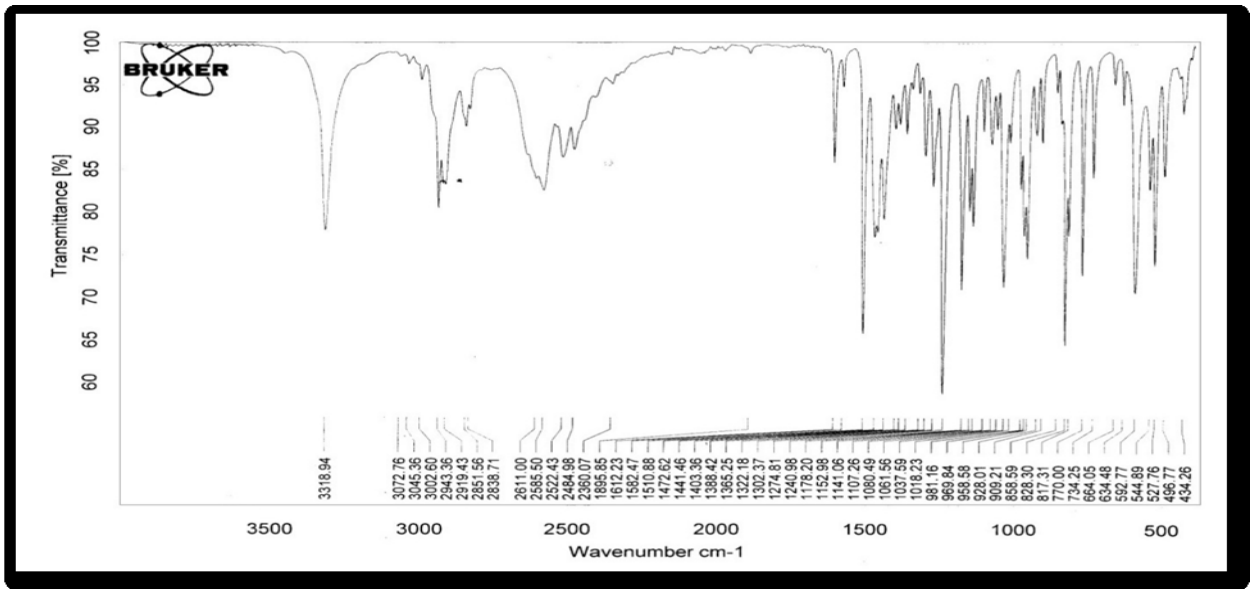
Table 5: Viscosity of All Formulation

Sr. No.	Temperature ^{0}C	Viscosity (cP)			
		A_1	**A_2**	**A_3**	**A_4**
1	28	170	165	230	250
2	30	320	280	310	390
3	32	430	360	460	580
4	34	580	580	590	810
5	36	640	690	800	1040
6	38	890	940	1120	1370



Graph 11: Viscosity of A_1, A_2, A_3, A_4 formulations with change in temperature at the rate of 2 ^{0}C.

IR spectrum for Venlafaxine Hydrochloride:

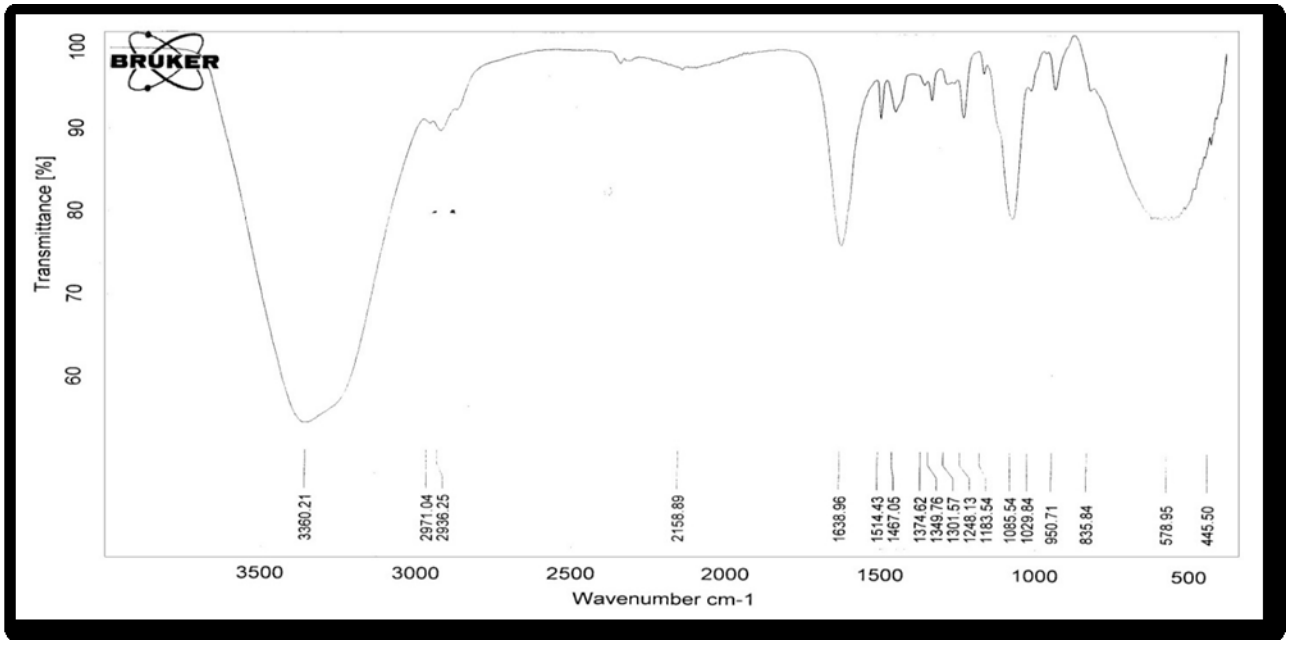


Graph 12: IR spectrum of Venlafaxine Hydrochloride

Table 6: Interpretation of IR spectrum of Venlafaxine Hydrochloride

Functional group	Vibrational Frequencies	
	Observed	Reported
OH alcoholic Stretching	3318.94 cm^{-1}	3600-3300cm^{-1}
C-H aromatic ring	3045.36 cm^{-1}	3050cm^{-1}
C-H alkanes	2919.43 cm^{-1}	2960 cm^{-1}
3 ^{0} amine	1322.18 cm^{-1}	1360-1310 cm^{-1}
C-N 3 ^{0} amine	1080 cm^{-1}	1250-1020 cm^{-1}
C-O-C (ether)	1037.59 cm^{-1}	1075-1020 cm^{-1}

IR spectrum for A2 formulation:

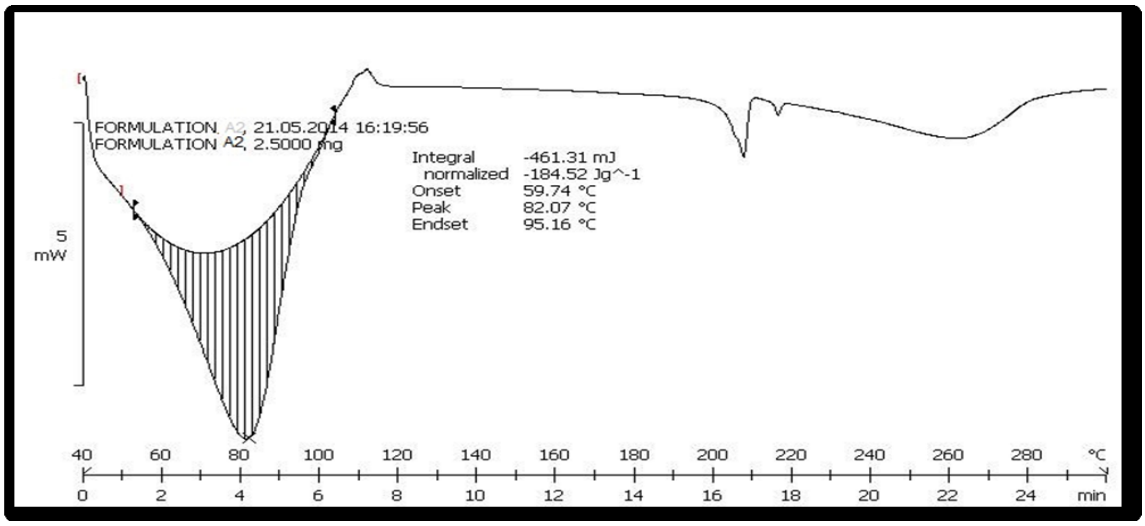


Graph 13: IR spectrum of A2 Formulation

Table 7: Interpretation of IR spectrum of A2 Formulation

Functional group	Vibrational Frequencies	
	Observed	Reported
OH alcoholic Stretching	3360.21 cm^{-1}	3600-3300cm^{-1}
C-H	2936.25 cm^{-1}	2960 cm^{-1}
C=O in polymer	1638.96 cm^{-1}	1660 cm^{-1}
3 ^{0} amine	1349.76 cm^{-1}	1360-1310 cm^{-1}
C-N 3 ^{0} amine	1085.54 cm^{-1}	1250-1020 cm^{-1}
C-O-C (ether)	1029.84 cm^{-1}	1075-1020 cm^{-1}

DSC of A2 formulation:

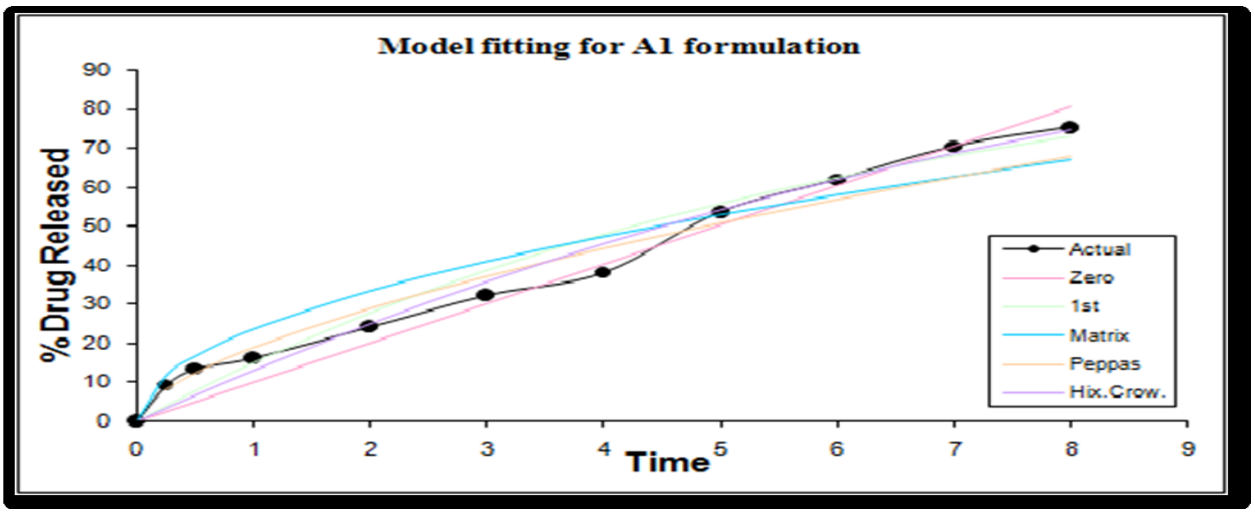


Graph 14: DSC of A2 formulation

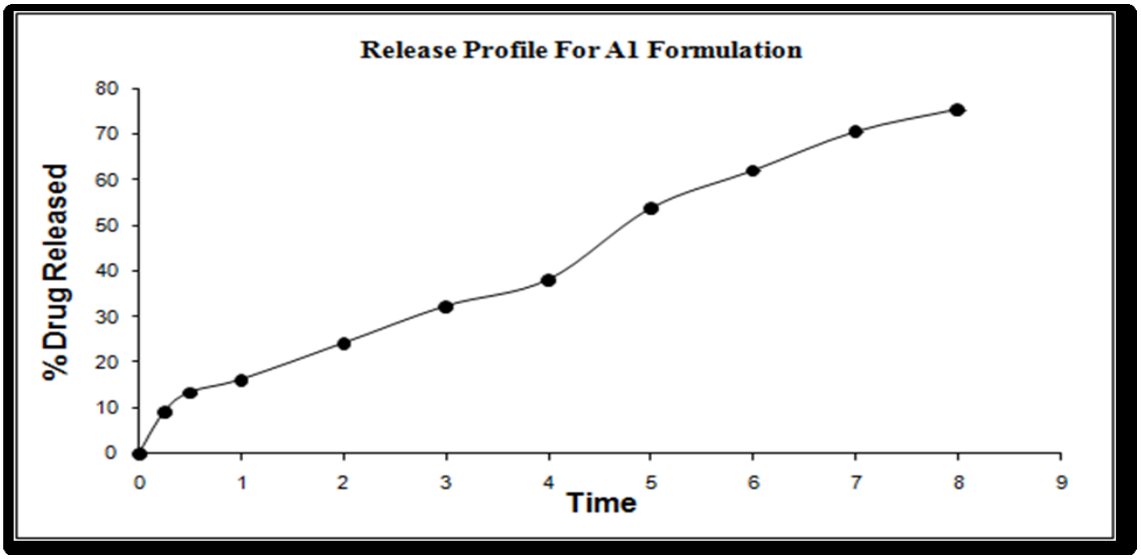
Diffusion study of A1 formulation:

Table 8: Ex-vivo diffusion study of A1 formulation

Times (hr)	Absorbance	Conc. (µg/ ml)	Conc. (µg/ 10ml)	Conc. (mg / 0.5 ml)	Conc. (mg/60 ml)	CDD	% CDD	t 50
0	0	0	0	0	0	0	0	4.5 hr
0.25	0.095	2.968	29.687	0.029	1.781	1.781	8.906	
0.5	0.142	4.437	44.375	0.044	2.662	2.692	13.460	
1	0.172	5.375	53.75	0.053	3.225	3.269	16.346	
2	0.26	8.125	81.25	0.081	4.875	4.928	24.643	t 90
3	0.349	10.906	109.062	0.109	6.543	6.625	33.125	
4	0.411	12.843	128.437	0.128	7.706	7.815	39.076	
5	0.583	18.218	182.187	0.182	10.931	11.059	55.298	11.7 hr
6	0.671	20.968	209.687	0.209	12.581	12.763	63.817	
7	0.761	23.781	237.812	0.237	14.268	14.478	72.392	
8	0.81	25.312	253.125	0.253	15.187	15.425	77.126	



Graph 15: Model fitting for A1 formulation

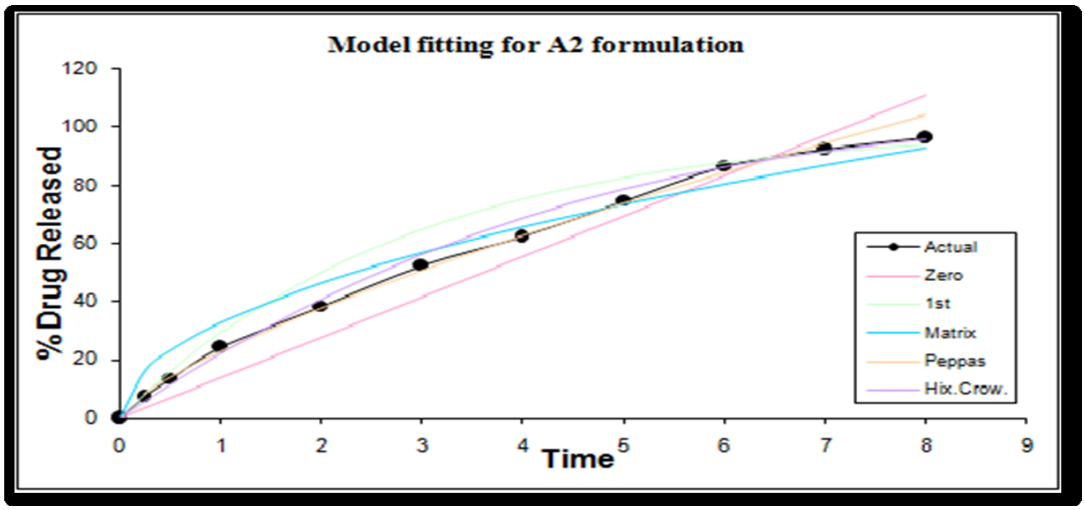


Graph 16: Release profile for A1 formulation

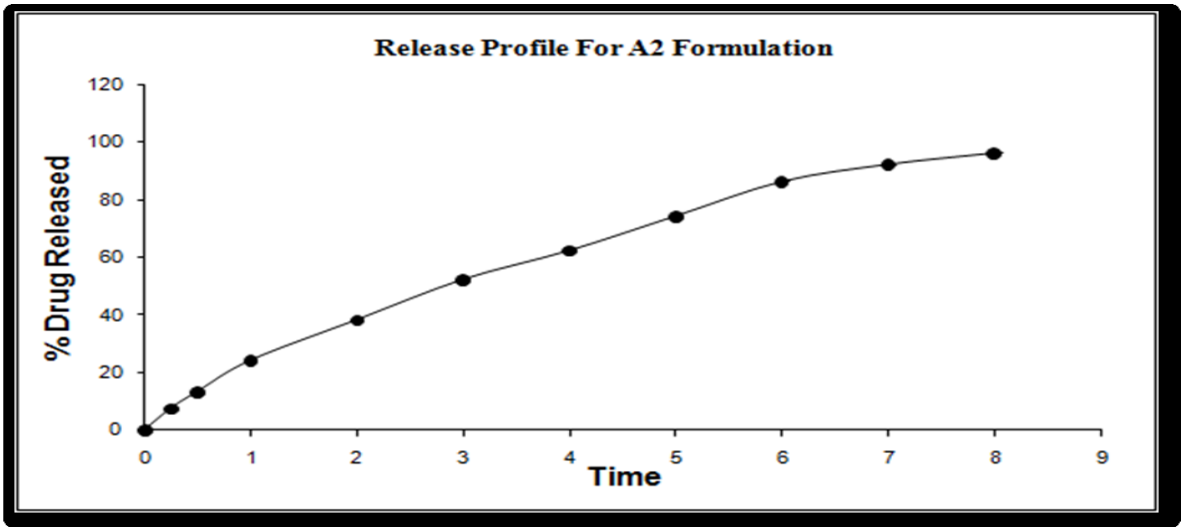
Diffusion study of A2 formulation:

Table 9: Ex-vivo diffusion study of A2 formulation

Times (hr)	Absorbance	Conc. (µg/ml)	Conc. (µg/ 10ml)	Conc. (mg/ 0.5 ml)	Conc. (mg/60 ml)	CDD	% CDD	t 50
0	0	0	0	0	0	0	0	3.0
0.25	0.077	2.406	24.062	0.024	1.443	1.443	7.218	hr
0.5	0.141	4.406	44.062	0.044	2.643	2.667	13.339	
1	0.262	8.187	81.875	0.081	4.9125	4.956	24.782	
2	0.418	13.062	130.625	0.130	7.8375	7.919	39.596	t
3	0.572	17.875	178.75	0.178	10.725	10.855	54.278	90
4	0.68	21.25	212.5	0.212	12.75	12.928	64.643	
5	0.809	25.281	252.812	0.252	15.168	15.381	76.906	6.6
6	0.937	29.281	292.812	0.292	17.568	17.821	89.107	hr
7	0.996	31.125	311.25	0.311	18.675	18.967	94.839	
8	1.032	32.25	322.5	0.322	19.35	19.661	98.306	



Graph 17: Model fitting for A2 formulation

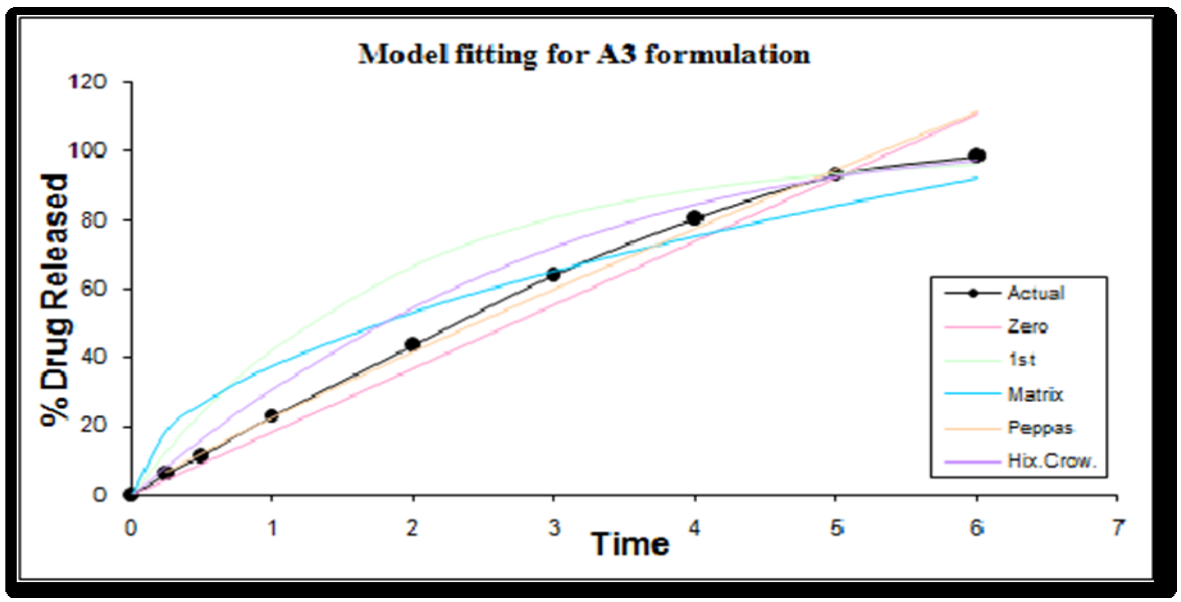


Graph 18: Release profile for A2 formulation

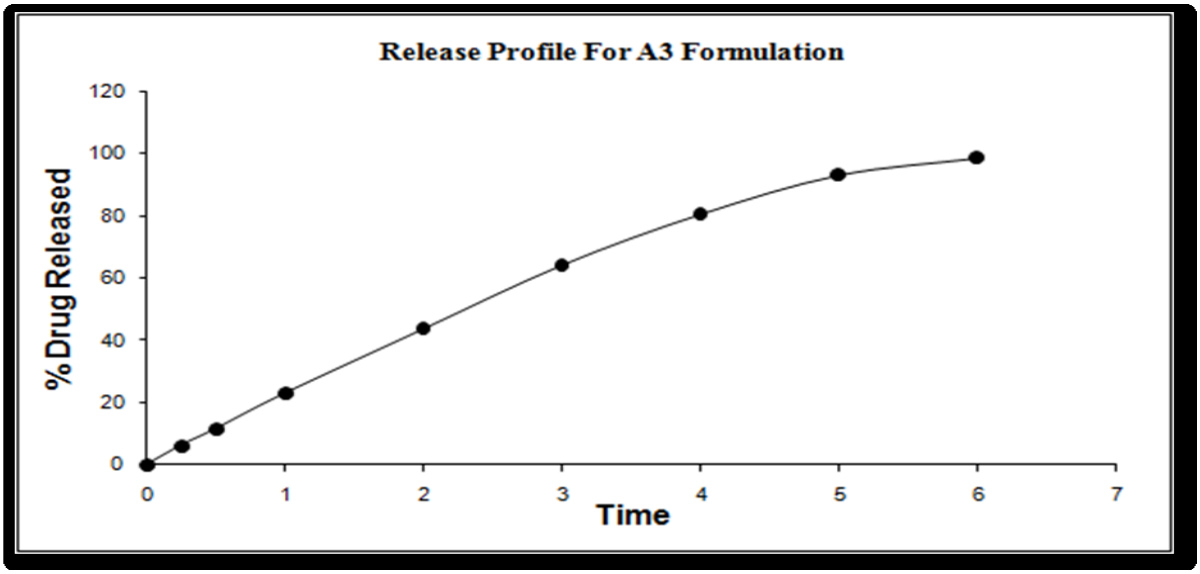
Diffusion study of A3 formulation:

Table 10: Ex-vivo diffusion study of A3 formulation

Times (hr)	Absorbance	Conc. (µg/ml)	Conc. (µg/ 10ml)	Conc. (mg/ 0.5 ml)	Conc. (mg/60 ml)	CDD	% CDD	t 50
0	0	0	0	0	0	0	0	
0.25	0.062	1.937	19.375	0.019	1.162	1.162	5.812	2.5
0.5	0.121	3.781	37.812	0.037	2.268	2.288	11.440	hr
1	0.248	7.75	77.5	0.077	4.65	4.687	23.439	
2	0.478	14.937	149.375	0.149	8.962	9.04	45.2	t
3	0.704	22	220	0.22	13.2	13.349	66.746	90
4	0.882	27.562	275.625	0.275	16.537	16.757	83.787	
5	1.016	31.75	317.5	0.317	19.05	19.325	96.628	4.7
6	1.045	32.656	326.56	0.326	19.593	19.911	99.556	hr



Graph 19: Model fitting for A3 formulation

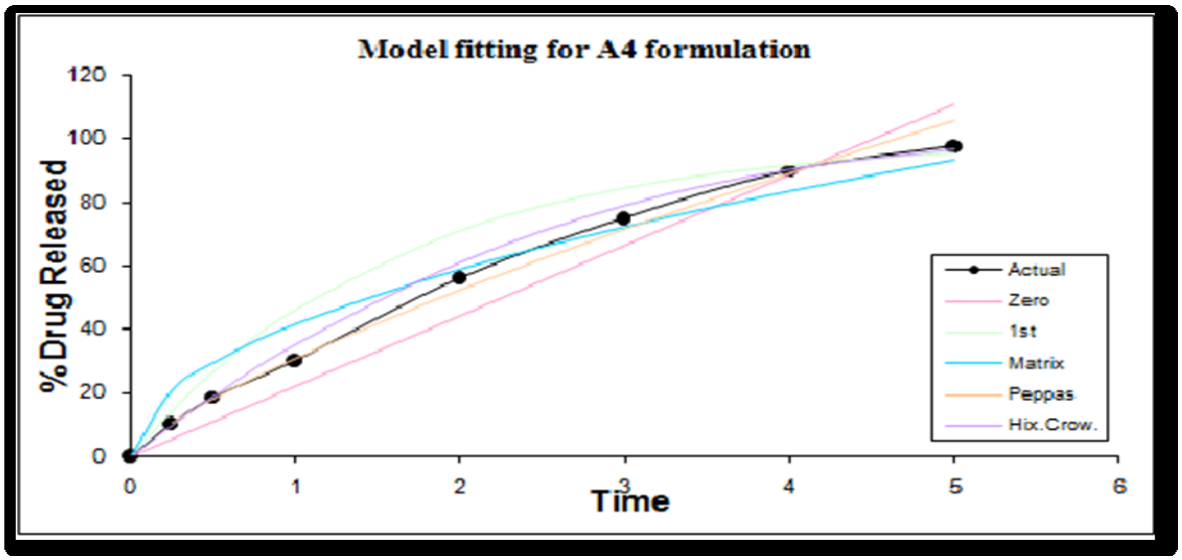


Graph 20: Release profile for A3 formulation

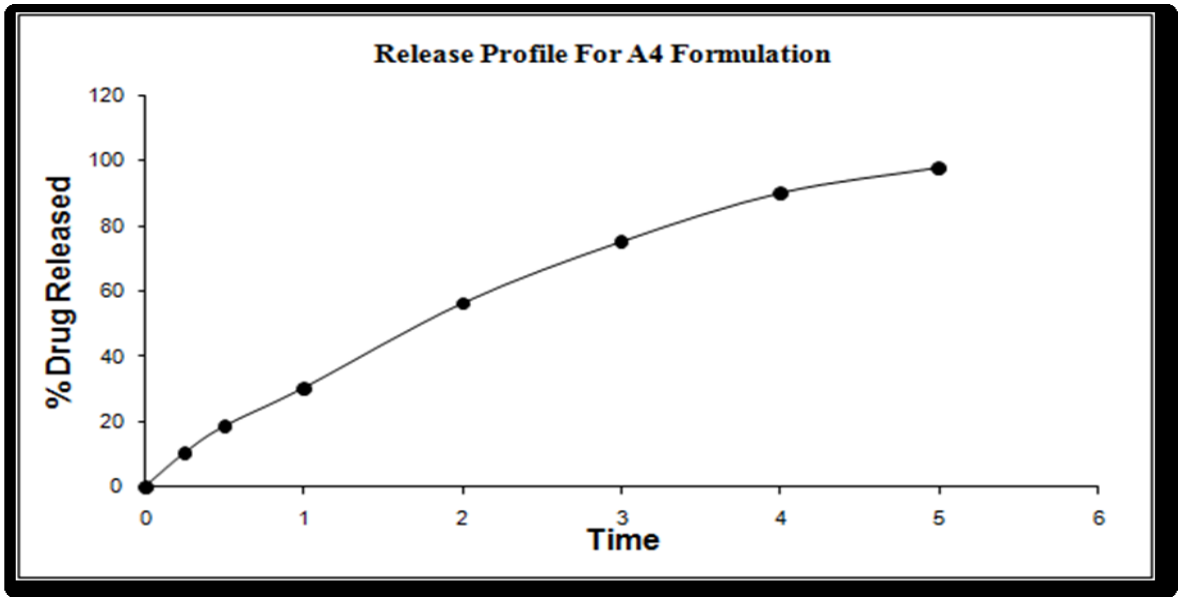
Diffusion study of A4 formulation:

Table11: Ex-vivo diffusion study of A4 formulation

Times (hr)	Absorbance	Conc. (µg/ml)	Conc. (µg/ 10ml)	Conc. (mg/ 0.5 ml)	Conc. (mg/60 ml)	CDD	% CDD	t 50
0	0	0	0	0	0	0	0	
0.25	0.109	3.406	34.062	0.034	2.043	2.043	10.218	1.9
0.5	0.199	6.218	62.187	0.062	3.731	3.765	18.826	hr
1	0.329	10.281	102.812	0.102	6.168	6.230	31.154	t
2	0.617	19.281	192.812	0.192	11.568	11.671	58.357	90
3	0.823	25.718	257.187	0.257	15.431	15.624	78.120	
4	0.983	30.718	307.187	0.307	18.431	18.688	93.442	4.0
5	1.025	32.031	320.312	0.320	19.218	19.525	97.629	hr



Graph 21: Model fitting for A4 formulation

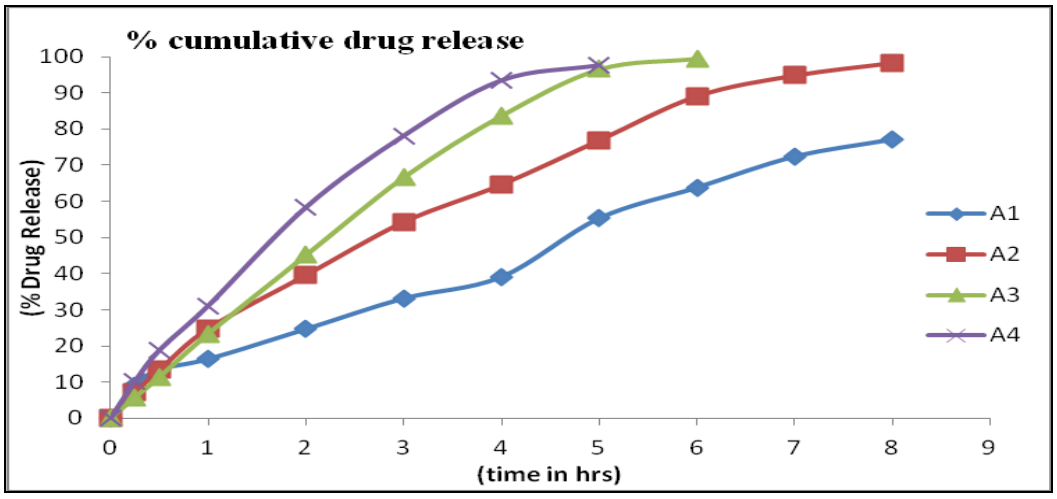


Graph 22: Release profile for A4 formulation

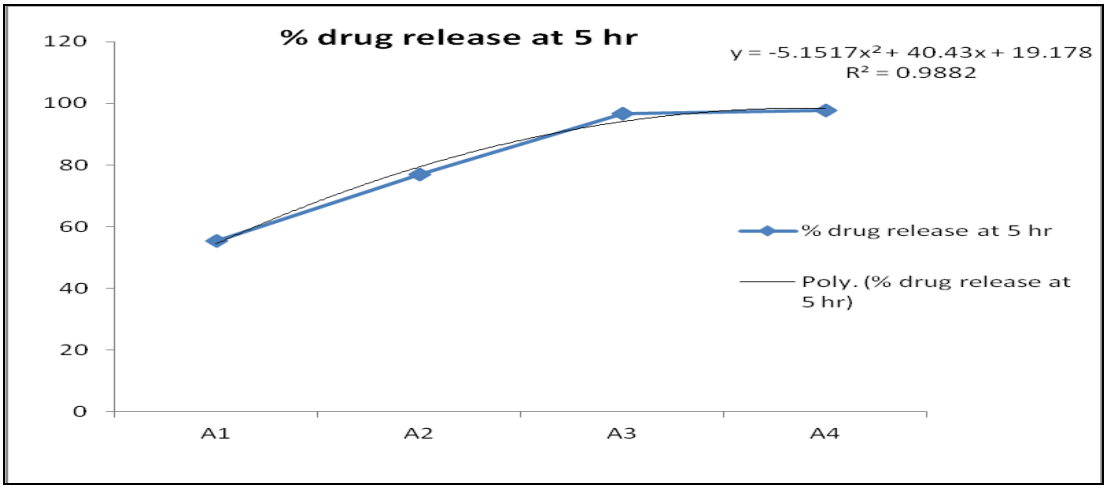
% cumulative drug release of all formulations

Table 12: % Cumulative Drug Release of all Formulations of Venlafaxine Hydrochloride

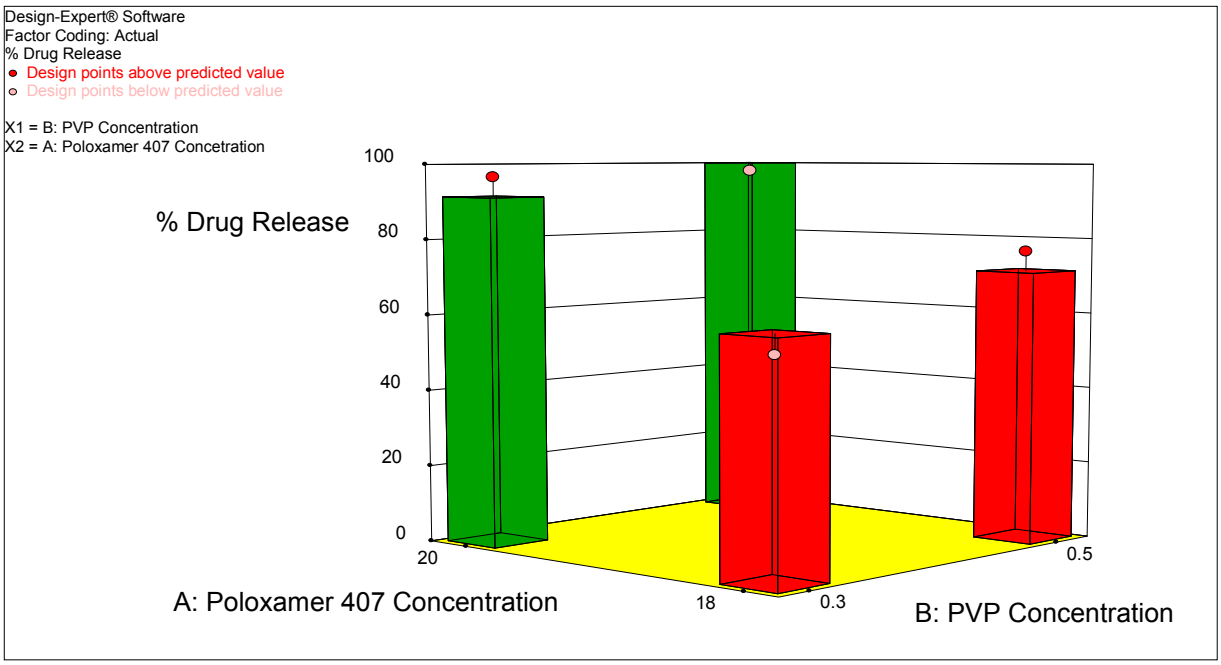
Time (hr)	A1	A2	A3	A4
0	0	0	0	0
0.25	8.906	7.218	5.812	10.218
0.5	13.460	13.339	11.440	18.826
1	16.346	24.782	23.439	31.154
2	24.643	39.596	45.2	58.357
3	33.125	54.278	66.746	78.120
4	39.076	64.643	83.787	93.442
5	55.298	76.906	96.628	97.629
6	63.817	89.107	99.556	-
7	72.392	94.839	-	-
8	77.126	98.306	-	-



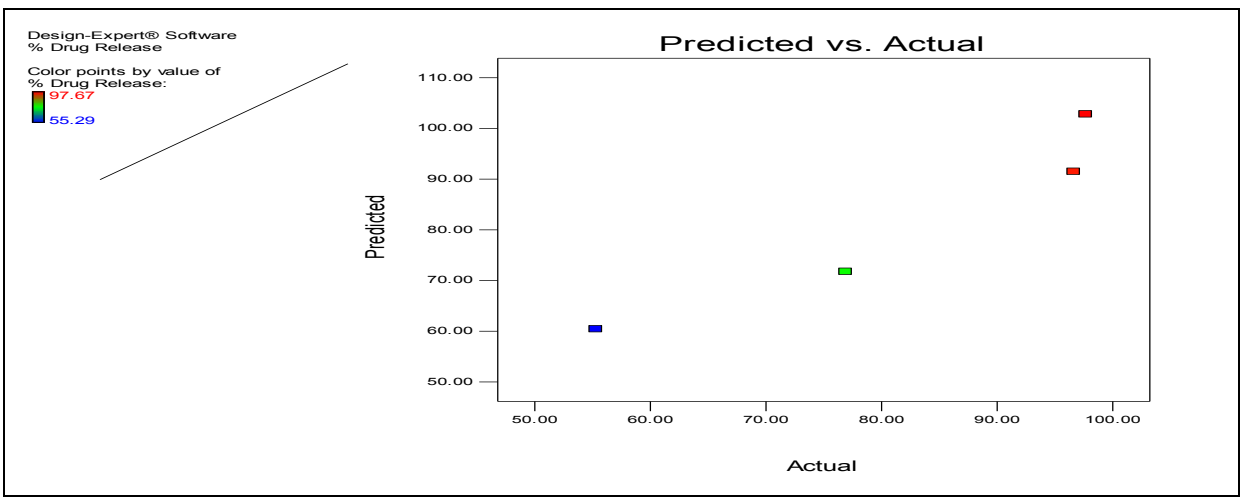
Graph 23: % Cumulative Drug Release of all Formulations of Venlafaxine Hydrochloride



Graph 24: Polynomial equation for % Drug Release



Graph 25: Effect of main factor on % Drug Release presented by 3D surface plot of Poloxamer 407: Polyvinylpyrrolidone



Graph 26: Effect of main factor on % Drug Release presented by linear plot of Poloxamer 407: Polyvinylpyrrolidone

Data Analysis of Release kinetics:

Table 13: Best model fit for all formulations of Venlafaxine Hydrochloride

Formulation	Zero Order	First Order	Higuchi Matrix	Peppas Plot	Hixson Crowell	'n' Values	Best fit Model
A1	0.9838	0.9853	0.9648	0.9839	0.9913	0.6149	Hixson
A2	0.9710	0.9685	0.9828	0.9986	0.9956	0.7381	Peppas
A3	0.9855	0.9371	0.9641	0.9982	0.9879	0.8965	Peppas
A4	0.9727	0.9604	0.9778	0.9983	0.9956	0.7639	Peppas

Stability Study:

Table 14: Drug content, pH and Viscosity of Formulation A_2 during stability study at 40 ± 0.5°C; 75%5% RH condition.

Time in Days	Drug Content (40 ± 0.5 °C; 75% 5% RH)	pH (40 ± 0.5 °C; 75% 5% RH)	Viscosity at 36 ^{0}C (cP) (40 ± 0.5°C; 75% 5% RH
0	98.35±0.12	6.8±0.05	680±0
7	98.56±0.34	6.8±0.15	690±0
14	98.34±0.28	6.7±0.1	670±5.7
21	97.48±0.21	6.8±0.11	680±10
28	97.23± 0.34	6.6±0.05	700±10
35	97.35± 0.22	6.7±0.11	690±5.7
42	97.17± 0.41	6.6±0.5	670±5.7

DISCUSSION

The main goal of this work was to develop new thermoreversible in situ nasal gel of Venlafaxine Hydrochloride, an anti-depressant drug. Total 4 formulations of thermoreversible in situ nasal gel of Venlafaxine Hydrochloride were prepared and evaluated for biological, physical and mechanical parameters. According to work plan, the in-situ gel evaluated for their clarity, pH, drug content, gelation temperature, mucoadhesive strength, viscosity, IR spectroscopy, DSC, Ex-vivo diffusion study, data analysis and accelerated stability study The results of all these evaluations are given in tables-4 to 14.

pH

pH of all formulation were in the range of in the range of 6 -7.0 as shown in table no.4.

% Drug Content

The percentage drug content of all formulations was found to be in the range of 96-99.89% w/v. The percentage content of all formulations from the same batch was found to be uniform as shown in observation table no. 4. The change in drug content of each batch may be due to dilution error arises during the determination of drug content.

The polynomial equation for % Drug Content is as bellow with R coefficient

$Y = 0.217x^2 - 0.580x + 98.72$

$R^2 = 0.998$

The polynomial equation for gelation temperature was found in significant range. The multiple regression data obtained for % Drug Content indicated positive influence of the variables on the study. Graph of % drug content, polynomial equation, Effect of main factor on % drug content presented by 3D surface plot and Effect of main factor on % drug content presented by linear plot of % drug content are shown in graph no.6 to 9.

Gelation Temperature

The gelation temperature (T_g ^{0} C) of formulation were found within the 25-34 ^{0}C. The effect of polymer combination on gelation temperature of optimized Poloxamer 407 was as compared to individual polymer shown in observation table no.4. It was found that Venlafaxine Hydrochloride does not show any prominent effect on gelation of any formulation as shown in control, this might be due to the strong hydrogen bonding between hydroxyl groups of polymers and oxygen of polyethylene oxide of Poloxamer 407.

The polynomial equation for Gelation temperature is as bellow with R coefficient

$y = 3E\text{-}14x^2 - 1.6x + 38.5$

$R^2 = 0.984$

The polynomial equation for gelation temperature was found in significant range. The multiple regression data obtained for gelation temperature indicated positive influence of the variables on the study. Graph of Gelation Temperature, polynomial equation, Effect of main factor on Gelation Temperature presented by 3D surface plot and Effect of main factor on Gelation Temperature presented by linear plot of gelation temperature are shown in graph no.1 to 4.

Mucoadhesive Force

The stronger the bioadhesive force greater will be the nasal residential time. As the concentrations of polymers increased the mucoadhesion force was also increased. Batches coded as A_3 and A_4 showed better mucoadhesive strength. From observation table no. 4 and graph no.5 it has been observed that all mucoadhesive polymers under study show increase in mucoadhesion as compared to control Poloxamer 407 solution. From the various study it has been found that the presence of polyoxyethylene groups in Poloxamer 407 responsible for the hydrogen bonding with mucosa components. But in case of thermoreversible gel formulation free polyoxyethylene groups are may be unavailable for hydrogen bonding due to increased cross linkage between the Poloxamer 407.

Viscosity

The study of the rheological behavior of liquid and semi-solid pharmaceutical formulations is important in view of their complex nature and their possible influence on manufacturing processes. Poloxamer 407 solution has unusual rheological characteristics including thermoreversible gelling, gel-sol transition temperature and viscosity, which have to be studied. The polymer solution is a highly viscous gel at room temperature, but becomes a liquid at low temperature (5 ^{o}C). The gel undergoes thermoreversible gelling and can be cooled and warmed many times without changing its properties. Viscosity studies were performed by changing the temperature and keeping the shear rate constant. The results obtained were given in observation table no 5 and graph no. 10.From the observation table it was observed that increase in polymers concentration results in decrease in gelation temperature and increase in viscosity. All formulation shows decrease in gelation temperature as increasing polymer concentrations. The order of increasing viscosity was found to be $A_1 < A_2 < A_3 < A_4$.

Differential Scanning Calorimetry (DSC) Study

The Differential Scanning Calorimetry (DSC) study for pure drug Venlafaxine Hydrochloride and selected formulation A2 were shown in graph no.13. The drug and its polymeric combination melts at the temperature 82 ^{0}C. The polymeric form of drug represent small blunt endothermic peak represent drug remain in less crystalline polymeric form, and sharp endothermic at temperature 207 ^{0}C indicate that drug present in more in amorphous polymeric form which help to exhibit more solubility of drug. Absence of any additional endotherm indicated that there was no any chemical interation between drug and excipient.

IR Spectroscopy

IR spectra of optimized formulations show in graph no. 12. The observed spectrum represent drug to polymer super-imposed pattern with their significant functional group at specific wave length indicated in its pure form. The spectrum represents significant functional group such as OH, 3^0 amine, C=O, C-N 3^0 amine and C-O-C

(ether). From drug to polymers, no new peak formation was observed indicated that there was no any chemical interaction between drug and polymers only the physical interaction was take place in terms of hydrogen bonding.

Ex- Vivo Diffusion Studies

Ex-Vivo diffusion studies for All Formulations carry out by using sheep nasal mucosa. Results obtained were represented in observation table no. 8 to 11. Percent cumulative drug release obtained from various formulations was given observation table no. 12. Best release kinetics fitting model to all formulation shows in table no. 13 Formulations A2, A3 and A4 best fitted into Peppas dissolution mathematical model while formulations A1 fitted into Hixson mathematical model.

The polynomial equation for % Drug Release is as bellow with R coefficient

$Y= -5.151x^2 + 40.43x + 19.17$

$R^2 = 0.988$

The polynomial equation for gelation temperature was found in significant range. The multiple regression data obtained for % Drug Release indicated positive influence of the variables on the study. Graph of % drug release, polynomial equation, Effect of main factor on % drug release presented by 3D surface plot and Effect of main factor on % drug release presented by linear plot of gelation temperature are shown in graph no23 to 26.

Data Analysis of Release Kinetics

A2 formulation shows Peppas and Higuchi Matrix dissolution mathematical models. 'n' value was 0.7381 of A2 formulation. Which indicate that A2 formulation shows the drug release by Anomalous transport (non-Fickian). This model is widely used; when the release mechanism is not well known or when more than one type of release phenomena could be involved. Anomalous transport means drug diffusion in the hydrated matrix and the polymer relaxation. The ex-vivo diffusion parameter values ($t_{50\%}$ and $t_{90\%}$) displayed by the A2 Formulation 3.0 hr ($t_{50\%}$) and 6.6 hr ($t_{90\%}$) respectively. The formulations A2 (containing Poloxamer 407 18% with 0.5 % PVP). From all above evaluation parameters formulation A2 was optimized formulation. Which was further studied for 45 days stability study.

Stability Study

Stability of pharmaceutical product may be defined as the capability of a particular formulation in a specific container closure system, to remain within its physical, chemical, microbiological, therapeutic and toxological specifications. There are mainly three types of stability testing mainly;

I. Long term stability testing: Conducted in laboratory under controlled stresses similar to those to be encountered during storage.
II. Field testing: Testing of packed material after transporting.
III. Accelerated stability study: The substance or medicinal product challenged by controlled, exaggerated stress over short time.

Accelerated stability study was selected for 45 days; sample was kept to 40±2 ^{0}C, 75±5% relative humidity represented stability study of optimized (A2) formulation exhibited the following observations also it is represented in observation table no. 14.

Observations of stability study:

I. Clarity and pH:
   Formulations kept for stability studies were examined. There was no any change in clarity and pH in the optimized formulations A_2 over a period of 42 days in accelerated and room temperature exaggerated conditions.
II. Viscosity:
   Viscosity was observed both formulations from A_2 batche for both condition show small change in Viscosity studied by viscometer. A formulation from A_2 batch shows 670 cP respectively. At room temperature viscosity was found 720.
III. % w/v Drug content:
   Drug content was determined after every specified interval of time in days for 7, 14, 21, 28, 35 and 42days. At the end of 42 days the drug content of A_2 batch for stability chamber condition was found to

be 98.50 ± 0.134 % w/v which is not significantly different from initial values. And at room temperature condition it shows 96.12 %. This indicates that Venlafaxine Hydrochloride was stable in presence of excipients used at accelerated temperature.

CONCLUSION

For the formulation of thermoreversible gel, Poloxamer 407 18% was used a thermoreversible gel-forming polymer as it formed gel at nasal physiological temperature. PVP was tried as mucoadhesive polymers in different concentrations. These formulations were tested for mucoadhesive strength by using sheep nasal mucosa. Depending upon mucoadhesive strength, formulations A1, A2, A3 and A4 were selected for further evaluation of gel strength, viscosity, drug content, and diffusion through sheep nasal mucosa. All four selected batches were found to be having almost same release profile, for 6 to 8 hr, i.e., 97.85±0.05 to 99.15±0.36% of drug release was achieved. Formulation A2 was selected as the final optimized formulation and used for Stability study. Thus, in the present study nasal drug delivery of Venlafaxine Hcl was successfully formulated in the form of thermoreversible nasal in situ gel which can be a good option for administration of the drug as well as it can also be thought of a drug delivery route for such CNS drugs for brain targeting.

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