AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Preparation, Evaluation and Optimization of In-situ gel of Fluoxetine HCl for Intranasal drug delivery

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ABSTRACT

In-situ gelling system is a process in which a solution form converted into the gel form after the formulation has been applied at the site. In-situ gel is helpful to produce sustained release of drug. Fluoxetine HCl is a selective serotonin reuptake inhibitor. The current study deals with the preparation, evaluation and optimization of In-situ gel of Fluoxetine HCl for intranasal delivery for the effective treatment of depression. The In-situ gel of Fluxetine HCl was prepared by cold-technique and evaluated for its physical appearance, viscosity, Gel forming temperature, Melting temperature, pH, Spreadability, Swelling index, Drug content and In-vitro release study. In-vitro drug release for optimized formulation was found to be 95.55% of F2 and pH, Drug content, Spreadability, Gelling temperature, Melting temperature were found to be 6.3, 97.6%, 8.0 gmcm/sec, 33^0C, 79^0C respectively. The study clearly demonstrated that the Fluoxetine HCl can be successfully delivered through nasal route by preparing in-situ gel. Gel is non-irritating when delivered through nasal route.

Keywords: Intransal delivery, Fluoxetine HCl, In-situ nasal gel

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Received 14 June 2016, Accepted 25 June2016

Please cite this article as: Mukhopadhyay S *et al.*, Preparation, Evaluation and Optimization of In-situ gel of Fluoxetine HCl for Intranasal drug delivery. American Journal of PharmTech Research 2016.

INTRODUCTION

Brain is the most important organ of human body which is protected by the two layers which are blood brain barrier and blood cerebrospinal fluid barrier. For treating brain diseases crossing of BBB is a major challenge. BBB is a protective layer of brain which protect from the toxic substances present in the blood stream. For the treatment of many CNS disorders such as brain and spinal cord injury, brain cancer, Alzheimer, Parkinson's, Depression, multiple sclerosis etc many novel delivery systems are developed.[1] Most of the drugs are effective at their site of action but in the case of CNS drugs proper quantity of drug is not able to reach the brain by which they are discarded in the phase of the development for the clinical use.[2] Blood brain barrier and cerebrospinal fluid barrier are two barrier which are responsible for the failure in the delivery of drug to the brain. In the delivery of CNS drugs it must be important that drugs should passes the BBB.[3] Nasal delivery of drug is the best way for direct drug delivery in the biophase of CNS active compounds because nasal delivery is able to bypass the BBB. It is also used for the administration of vaccines.[4,5]Mostly for the systemic action of drug transmucosal route is used. Nasal drug delivery is an effective way for the topical therapies as well as systemic therapies because nasal cavity have low enzymatic environment, high permeability and high vasculature so nose is suitable for delivery of drug molecules. [6,7] In the ayurvedic system of Indian medicines the nasal therapy is known as Nasaya karma.[8] Nasal application is a nonparenteral delivery system by which rapid onset of action is possible. By the nasal route only low molecular weight hydrophobic drugs are highly absorbed.[9]When the small molecular weight polar drugs, proteins and peptides are not administered by other route then, nasal route is preferred.[10]

MATERIALS AND METHOD

Organolaptic Properties

Organoleptic properties of ciprofloxacin was observed by physical and visual method.

Melting Point

The sample was loaded in to sealed capillary (melting point capillary) which was then placed in melting point apparatus. The sample was then heated and as the temperature increase the sample was observed to detect the phase change from solid to liquid phase. The temperature at which the phase changes occur gives the melting point.[11]

Calibration Curve

Standard stock solution of concentration1000µg/ml was prepared by dissolving accurately weighed 100mg of fluoxetine in 100 ml volumetric flask. From stock solution dilution having concentration

2,4,6,8,10,12,14,16,18 and 20µg/ml were prepared. Absorbance of these solution was measured at 261nm using UV Spectrophotometer against blank.Calibration curve shows the slope and regression coefficient

Solubility

Drugwas carried out in demineralized water. The excess drug were added gradually to 5ml of each solvent contained in 10ml glass vials and vials were sealed with rubber closures and alluminium seals. The vials were shaker for 12 hours in orbital flask shaker and allowed to equilibrate for 24 hours undisturbed. The solution containing excess of drug were centrifuged at 2200rpm for and minutes in ultra-centrifuge and filtered through whatman grade 5 filters. Liquotes of the filtrate were suitably diluted and dilution were analyzed on UV-visible spectrophotometer.[12]

Preparation Techniques for In-situ Gel

Cold Process

Gels were prepared on a weight basis using a cold process. Carefully weighed an amount of Pluronic 188 sufficient to yield 20% was slowly added to cold water (5˚C); constant stirring was maintained. Each dispersion was then refrigerated until a clear solution is formed (5hr's). Active substances that are insoluble in water are dissolved prior to addition in Ethanol, Isopropyl alcohol or Propylene glycol at 5˚C to form a homogeneous mass.[13]

Hot Process

Pluronic 188 dissolved in water approximately at 70˚C. Active substances that are insoluble in water are dissolved in Ethanol, Isopropyl alcohol orPropylene glycol at 70˚C and mixed with warm aqueous phase to form a homogeneous mass before addition. The gel forms when the solution cools to room temperature.[13]

Characterization of in-situ nasal gel

Determination of Gelation Temperature of Prepared In-situ Gel

Gelation temperature was determined by pouring 5ml of formulation in a 20ml of beaker containing magnetic bead and placing it on a magnetic stirrer. A thermometer was immersed in this gel which was heated at a rate of 2°C/ min with constant stirring at 20 rpm. When the bead stopped moving due to gelation, the temperature was recorded as the gelation temperature.

Determination of Gel Melting Temperature of Prepared In-situ Gel

After gelation temperature again the temperature increases the point reached when the gel again melts, the temperature at which the bead starts to move again was recorded as gel melting temperature.

Determination of Viscosity

Viscosity of different formulations was measured with the viscometer at different rpm and temperature using spindle PE. [136]

Spreadability

Spreadability measured on the basis of slip and drag characteristic of formulation. A 20cm long glass slide, fixed on a table. 2g of gel placed on the glass. The gel then kept between this slide and another glass slide having the same dimensions. A 500g weight placed on the top of the two slides for 5minute to remove air and to provide a uniform film of the gel between the slides. The top glass plate was then subjected to pull with 50g of weight tied on the upper slide at a distance of 7cm. Lesser the time taken by the slides to move the specified distance of 7cm the better the spreadability.[15]

Spreadability is calculated from the following formula

$S = M \cdot L / T$

Where,

S= spreadability (g.cm/ sec)

M= wt on upper slide (g)

L= Length moved by the glass slide (cm)

T= Time taken to separate the slides completely from each other (sec)

pH of in-situ gel

it is known that the normal physiological pH of nasal mucosa is 5.5-6.5, however the nasal mucosa can tolerate solutions within pH range of 3-10.[16]

Swelling Index

Gel was accurately weighted to 100mg,and it was kept in a petri dish and 50ml of phosphate buffer 6.8 was added to it. Then it kept aside for 12hrs. After 12hrs the weight of swollen gel was measured and swelling index of gel was calculated by following equation-

Swelling Index= $(W_2 - W_1 / W_1) . 100$

Where,

W_1= Initial wt of gel

W_2= Wt of swollen gel after 12 hrs.[17]

In-vitro diffusion study of prepared in-situ nasal gel of Fluoxetine HCl

Diffusion study was carried out by Franz diffusion cell at a temperature of 37.5°C at 50 rpm. Treated dialysis membrane was placed in the diffusion cell. Specified quantity of in-situ gel was kept in the donor compartment. At predetermined time point sampling was done. Amount of drug permeated was determined by UV spectrophotometer.

Dialysis membrane mounted on diffusion cell. Placed accurate amount of gel containing 20mg of Fluoxetine HCl in it. The temperature maintained at 34± 1°C. At predetermined time intervals 1ml of sample was withdrawn at a time and replenished with an equal amount of phosphate buffer 6.4. The sample were diluted and filtered. Absorbances of sample were determined at 250nm by UV.[18]

IN VITRO DRUG RELEASE KINETIC STUDY

Kinetic models are used to describe drug release pattern. So the model fitting analysis done by comparing the regression coefficient values of all kinetic equations.[19,20]

Zero Order Model

Dissolution of drugs from dosage forms that release the drug slowly and do not disaggregate can be identified by the equation:

Qo-Qt = K0t

Or Qt = Qo + Kot

Where, Qt is the amount of drug dissolved in time t

Qo is the initial amount of drug in the solution

Ko is the zero order release constant expressed in units of concentration/time.

The graph was plotted between the cumulative amount of drug released and time as a data obtained from in vitro drug release to study the release kinetics.

First Order Model

The release of drugs which follows the first order kinetics can be identified by the following equation:

Log C = logCo-Kt/2.303

Where, Co is the initial concentration of the drug

K is the first order rate constant and t is the time.

The graph was plotted between log cumulative % of drug remaining and time and a straight line was obtained with a slope of –K/2.303.

Sometimes this model can also be used to describe the absorption and/or elimination of some drugs.

Higuchi Model

The following equation is used to describe this model:

ft = Q= KH X t1/2

Where, KH is the higuchi dissolution constant

The graph was plotted between cumulative % drug release and square root of time.

This is a hypothesis based model which is as follows

i. Initial drug concentration in the matrix is much higher than drug solubility.
ii. Drug diffusion takes place only in one dimension.
iii. Drug particles are much smaller than system thickness.
iv. Matrix swelling and dissolution are negligible.
v. Drug diffusivity is constant.
vi. Perfect sink conditions are always attained in the release environment.

Korsmeyer – Peppas Model

The following equation is used to identify this model

Mt/M∞= Ktn

Where, **Mt/M∞** is a fraction of drug released at time t

K is the release rate constant and n is the release exponent.

The n value is used to characterize different release for cylindrical shaped matrices.

The data was plotted between log cumulative % drug release and log time.

Hixson-Crowell model

Hixson and Crowell (1931) recognized that the particles regular area is proportional to the cube root of its volume. They derived the equation:

WO-Wt 1/3=ķt

Where, W0 is the initial amount of drug in the pharmaceutical dosage form. Wt is the remaining amount of drug in the pharmaceutical dosage form at time t. and k (kappa) is a constant incorporating the surface-volume relation.

The equation describes the release from systems where there is a change in surface area and diameter of particles or tablets .To study the release kinetics, data obtained from *invitro* drug release studies were plotted as cube root of drug percentage remaining in matrix versus time.

In this analysis the models used are zero order, first order, Higuchi, Korsmeyer- Peppas and Hixon -Crowll. The values of r^2 was used to choose the best fit model for each formulation and n value from Korsmeyer- Peppas was used to observed the mechanism of release.

To predict the mechanism of diffusion release, the following equation used

$M^t/M = kt^n$

Where,

n= 0.5(fickian diffusion)

0.5< n <1.0(anomalous(non-fickian) diffusion)

n= 1.0 (case Ⅱ diffusion

RESULTS AND DISCUSSION

Fluoxetine HCl is a selective serotonin reuptake inhibitor antidepressant drug. For the effective treatment of depression intranasal route is the best route because there is no barriers are present and drug can directly penetrate in the brain.

For this purpose in-situ gel of Fluoxetine HCl was prepared with different polymers like HPMC, Pluronic 188 for intransal delivery. The prepared in-situ gel formulation was evaluated for the different parameters.

Table1:Organolaptic properties, melting point and solubility estimation of Fluoxetine HCl

S.No	**Organolaptic properties**	
1	Description	Solid
2	Colour	White
3	Odour	Characteristic
4	Taste	Bitter
Evaluation of melting point		
S.No	Drug	Melting point
1	Fluoxetine HCl	158.6°C
Evaluation of solubility		
S.No	Solvent	Solubility
1	Methanol	Sparingly soluble
2	Water	Sparingly soluble
3	Phosphate buffer 6.4	Sparingly soluble

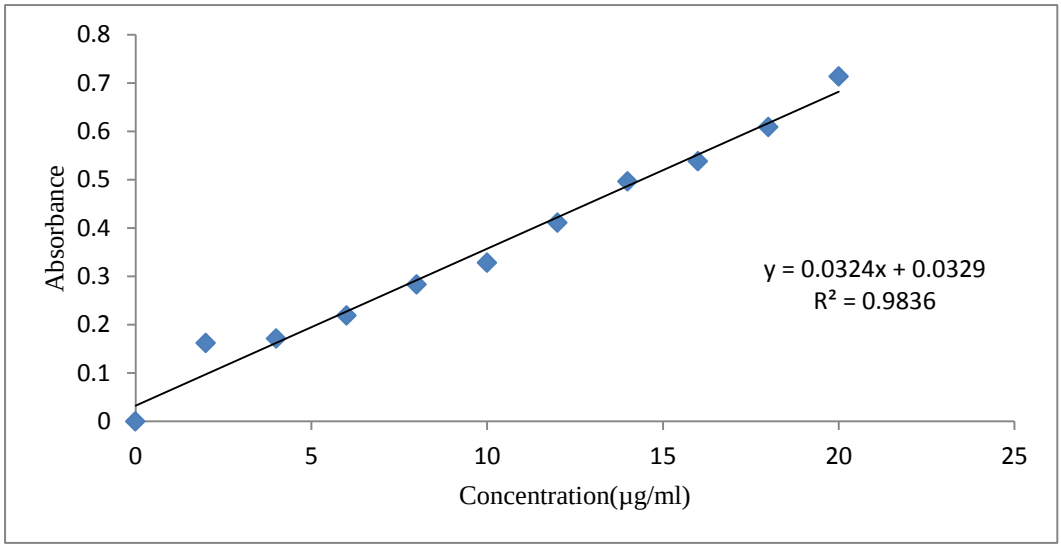

Figure 1: Calibration curve of Fluoxetine HCl in Methanol

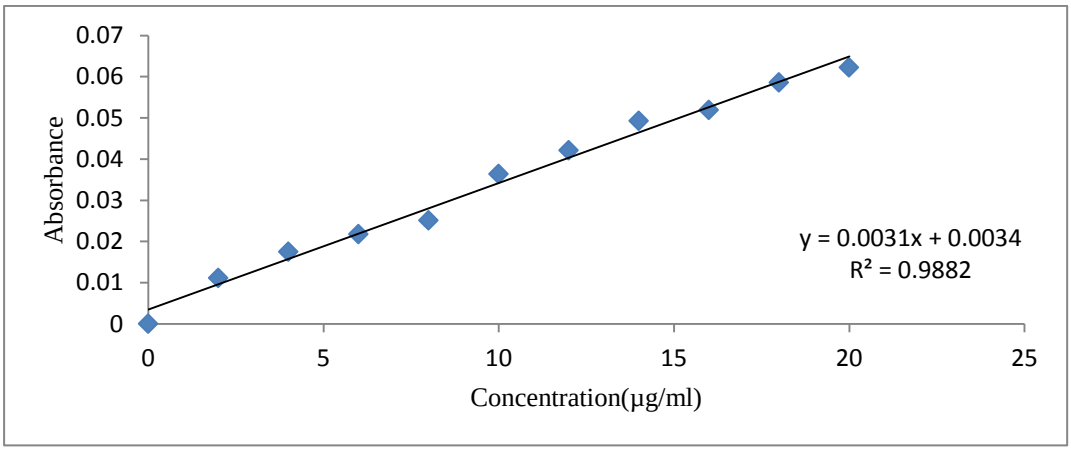

Figure 2: Calibration curve of Fluoxetine HCl in Water

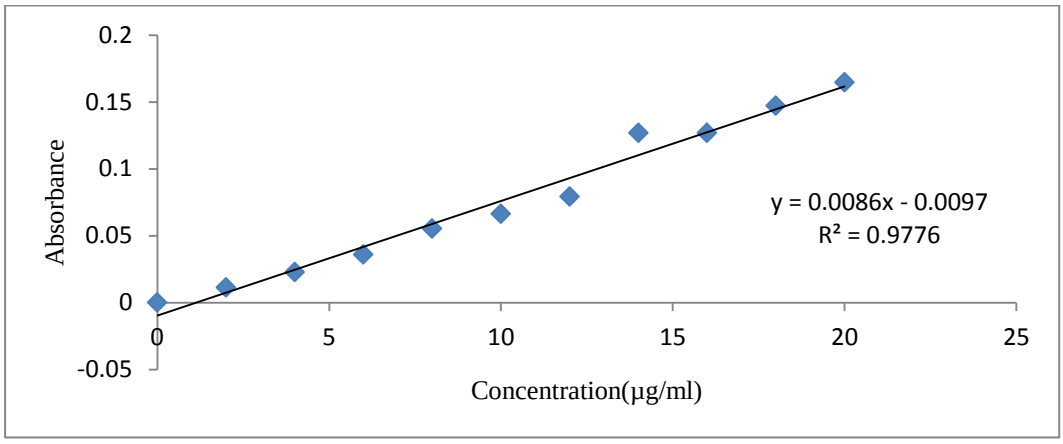

Figure 3: Calibration curve of Fluoxetine HCl in Phosphate buffer 6.4

Calibration curve of fluoxetine HCl was prepared in methanol, water and phosphate buffer 6.4. the calibration curve was plotted taking an absorbance on y-axis against concentration on x-axis.

Table2: Composition Table for In-situ gel of Fluoxetine HCl

Ingredients	F1	F2	F3	F4	F5	F6
Fluoxetine Hcl	20g	20g	20g	20g	20g	20g
Pluronic F188(w/v)	30%	30%	30%	30%	30%	30%
HPMC K4M(w/v)	0.1%	0.2%	0.3%	0.4%	0.5%	0.6%
Propylene glycol(v/v)	20%	20%	20%	20%	20%	20%
Benzalkonium chloride(v/v)	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Distilled water	100ml	100ml	100ml	100ml	100ml	100ml

Table3: pH, spreadability, swelling index, drug content of all prepared formulations

Formulation code	pH± SD	Spreadability± SD	Swelling index± SD	Drug content± SD
F1	6.7±0.12	10.42±0.38	6.76± 0.680	95.99± 0.9250
F2	6.3±0.08	8.00±0.320	4.7± 0.624	97.6± 0.6557
F3	6.5±0.06	7.59±0.09	5.93± 0.351	93.93± 0.777
F4	6.4±0.14	6.81±0.07	6.43± 0.416	90.77± 0.4703
F5	6.0±0.03	6.56±0.10	8.63± 0.472	92.32± 0.34
F6	6.5±0.21	5.94±0.06	7.5± 0.3	88.98± 0.4272

The pH was measured with the pH meter and all formulation's pH was within the range of 4.5-6.5, which is required for the nasal drug delivery system. For all formulations there is no significant change in their pH so these pH values are favourable for formulation. This neutral pH poses non-irritancy in nasal mucosa.

By evaluating the spreadability of in-situ nasal gel of Fluoxetine HCl it is clearly observed that as the concentration of polymer increased the spreadability is decreased. The values indicate that the in-situ nasal gel has good spreadability which is desired for the application of in-situ nasal gel.

As the concentration of swelling polymer increased the swelling index is also increased which signify better control release of formulation.

Drug content of prepared formulations were observed with the help of UV spectrophotometer. Experimental results suggest all the formulation having higher value of drug content within the range of 88.98-97.6%.

Gel forming temperature and gel melting temperature of all prepared formulations

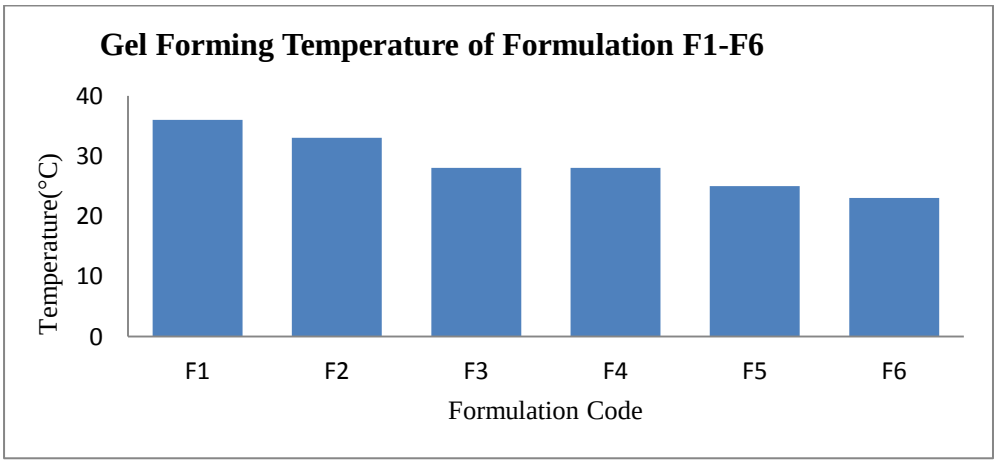

Figure 4:Gel forming temperature graph of all prepared Formulation

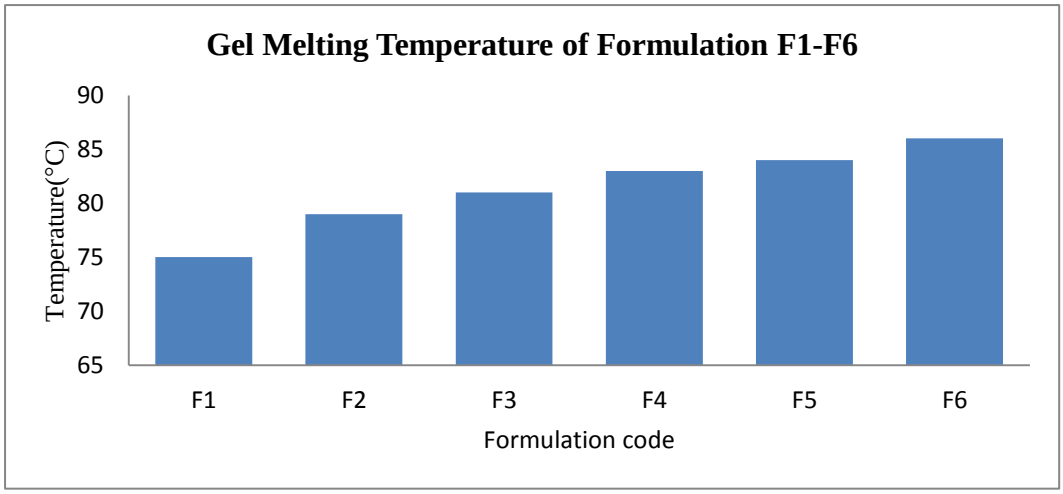

Figure 5: Gel melting temperature graph of all prepared Formulation

As the concentration of mucoadhesive polymer increased the gel forming temperature is decreased but all the values are within the range 23 to 36°C and as the concentration of mucoadhesive polymer increase the gel melting temperature is also increased.

Viscosity determination of all prepared formulation

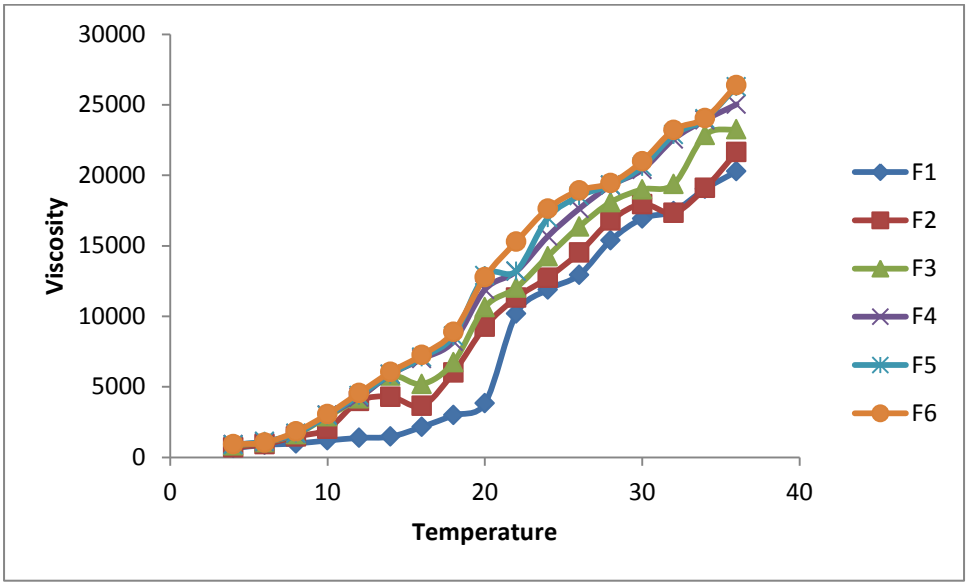

Figure 5:Graph between viscosity v/s temperature of all prepared formulation

When the viscosity measured against temperature and it was clearly observed that with the increasing temperature the viscosity of gel was also increase. This confirms the in-situ characteristics of prepared formulations. The orientation of graph between RPM and Torque confirm that gel follow the dilatant flow.

Table4: Cumulative percent release of all prepared formulations

Time	F1	F2	F3	F4	F5	F6
15	12.69±0.15	9.71±0.22	8.49±0.374	6.31±0.17	5.21±0.041	3.67±0.70
30	21.26±0.18	15.65±0.18	13.27±0.30	12.46±0.35	10.25±0.11	9.88±0.06
45	36.48±0.30	27.46±0.17	24.46±0.19	20.06±0.02	19.19±0.01	16.39±0.51
60	43.86±0.17	35.55±0.32	35.61±0.08	29.55±0.08	27.28±0.25	26.74±0.16
90	57.87±0.04	46.49±0.40	42.54±0.38	38.69±0.05	33.35±0.29	39.82±0.17
120	62.79±0.24	54.51±0.26	50.48±0.17	46.37±0.39	40.2±0.262	46.73±0.09
150	70.75±0.12	66.28±0.24	59.61±0.11	58.44±0.04	50.42±0.04	57.69±0.12
180	79.87±0.16	73.47±0.27	71.47±0.21	65.52±0.35	62.23±0.15	66.63±0.24
210	85.6±0.13	81.32±0.03	82.58±0.15	76.45±0.21	74.29±0.21	75.54±0.16
240	90.7±0.36	89.46±0.23	87.46±0.17	84.58±0.13	83.14±0.075	80.58±0.33
270	93.78±0.14	92.74±0.06	90.46±0.07	88.32±0.55	87.35±0.05	83.18±0.043
300	97.95±0.05	95.55±0.072	93.99±0.01	93.81±0.045	91.02±0.056	89.74±0.055

In-vitro release profile of in-situ nasal gel of Fluoxetine HCl was studied by using Franz diffusion cell. For this dialysis membrane with mil wt 70 was used. From the observed data it was clearly observed that released rate of cumulative percentage drug release were directly related to the

nature of polymer and its amount. From the prepared formulations it was observed that as the concentration of polymer increased (from 0.1 to 0.6%) the diffusion of drug from the polymer matrix is decreased, which slower the release rate. Formulation with lower polymer concentration (0.1%) released more amount of drug (97.95%) and with higher ratio (0.6%) showed slow drug release (89.74%) during study period. Not only the cumulative percentage drug release but release rate of drug was also significantly affected by polymeric concentration

Table5: Regression coefficient of Fluoxetine HCl for all prepared formulations

Formulation code	r^2				n*	Best fit model	Mechanism of release
	Zero order	1st order	Higuchi Matrix	HixonCrowll			
F1	0.918	0.945	0.987	0.992	0.664	Higuchi matrix	Non-Fickian
F2	0.96	0.967	0.982	0.996	0.774	Hixon- Crowll	Non-Fickian
F3	0.966	0.971	0.973	0.992	0.819	Hixon- Crowll	Non-Fickian
F4	0.982	0.955	0.967	0.990	0.894	Hixon- Crowll	Case 2
F5	0.970	0.955	0.950	0.982	0.945	Hixon- Crowll	Supercase 2
F6	0.988	0.986	0.964	0.996	1.034	Hixon- Crowll	Supercase 2

Kinetic models describe from immediate release and modified release dosage forms. Kinetic models describe drug release from immediate and modified release dosage forms. Thus the model fitting analysis (Zero Order, Higuchi, Hixon Crowell, First Order and Korsmeyer – Peppas Model) were done by comparing the coefficient of regression (r2) values and corresponding *n* value of all the kinetic equation. The correlation coefficient (r) values were used as criteria to choose the best model for the drug release from the in-situ nasal gel. For the in-vitro kinetic study the value of r^2 is used to choose the best fit model for the each formulation. The n value of Korsmeyer-Peppas used to observed the release mechanism.

From the kinetic models it is observed that the Hixon-Crowll model is best fit for the release of Fluoxetine HCl from in-situ gel and the mechanism of release is non-fickian.

Except formulation F1 all formulation Hixon-Crowll model was dominant. This kinetic model suggest that diffusion occurs in the plane that are parallel to the drug surface, if the gel dimensions diminish proportionally, in such a manner that the initial geometrical forms keep constant all the time. Except formulation F6 all the formulation follow non-fickian mechanism of drug release from initially dry hydrophilic glassy polymers that swell in contact of water and become rubbery show anomalous diffusion as a result of the rearrangement of macromolecular chains.

CONCLUSION

This study clearly demonstrated that the Fluoxetine HCl can be successfully delivered through

nasal route by preparing in-situ nasal gel. The gel was non-irritating and when it delivered through nasal mucosa provides an added advantage of bypassing the hepatic first pass metabolism. Well defined residence time of the in-situ gel in the nasal cavity, provide potential therapeutic benefit. It is possible to control the depression at faster rate by administrated Fluoxetine HCl into the nasal cavity which may transport the drug via olfactory epithelium directly into Central nervous system, by bypassing Blood brain barrier which increase its therapeutic concentration at targeted site.

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