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"THERMOSENSITIVE MUCOADHESIVE IN SITU GEL FOR INTRANASAL DELIVERY OF ATOMOXETINE HCl: FORMULATION, CHARACTERIZATION, AND EVALUATION"

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ABSTRACT:

In situ gels have been considered as promising drug delivery system for Atomoxetine, since they have been shown to sustain the drug release up to 7 hours. The main objective of this study was to prepare and characterize in situ gel of Atomoxetine HCl. Gels were prepared using Ploxamer 407 as thermosensitive polymer and HPMC K4M, HPMC K100 and Carbopol as mucoadhesive polymer using the cold method. Central composite experimental design was employed for the optimization of the effect of independent variables on the response (gelation temperature, mucoadhesive strength and % drug diffusion). Gels were evaluated for appearance, mucoadhesive strength, phase transition, and their performance in vitro and ex vivo using Franz diffusion cell. Acording to the findings of this research, an in situ gel of Atomoxetine HCl could be considered as a conceivable drug delivery system for treatment of ADHD.

Keywords: Atomoxetine HCl, ADHD, Intra nasal, In situ gel, Poloxamer 407, Thermo sensitive, Mucoadhesive.

1. INTRODUCTION

The nasal cavity has emerged as an appealing route for administering a wide range of medications via multi-site targeting, from small compounds to biopolymers [1]. The nasal route is a natural choice for administration of drugs topically. Drugs intended to treat disorders affecting sinuses and nose, such as infectious or allergic rhinitis, sinusitis, nasal sinusitis, and nasal sinus lesions [2]. In addition, the nasal mucosa is a non-invasive alternate route for systemic administration for low-bioavailability drugs. In fact, huge surface area existing for drug absorption due to covering of the epithelial exterior by abundant microvilli and the extremely vascularized nasal epithelium has been used to achieve rapid absorption of medicines that ordinarily undergo first-pass metabolism and/or stomach degradation after oral administration [3]. As well as absence of pancreatic and gastric enzymatic activity, neutral pH and minimum dilution by gastrointestinal contents are responsible for less degradation and loss of drug substances as compared to gastrointestinal rout. Also, nasal pathway may also be beneficial for delivering drugs to the brain, bypassing the blood-brain barrier (BBB)

which restricts diffusion transport mechanism of some therapeutic agents after oral or parenteral administration. Delivery from nose to brain ensures direct and rapid transfer of the medication from nasal cavity to central nervous system via the olfactory epithelium [4].

ADHD (Attention deficit hyperactivity disorder) is a chronic condition characterized by attention difficulty, hyperactivity and impulsiveness. It often begins in childhood and can persist into adulthood. It may contribute to low self-esteem, troubled relationships and difficulty at school or work [5].

ADHD is often associated with symptoms like hyperactivity, aggression, excitability, fidgeting, impulsivity, irritability, lack of restraint, or persistent repetition of words or actions, absent-mindedness, difficulty focusing, forgetfulness, problem paying attention, or short attention span, mood swings and depression or learning disability [5].

Atomoxetine HCl is the first non-stimulant drug approved for the treatment of ADHD and is classified as a selective norepinephrine reuptake inhibitor with 63% of oral absolute bioavailability in extensive metabolizers due to first pass metabolism by the Cytochrome P450 2D6 (CYP2D6) enzymatic pathway [6].

Hyperactivity and nonobedience ascribable to ADHD urge for alternative to conventional tablet formulations. Parenteral dosing, one of the alternatives, involves obvious inconvenience

Even though the nasal route proposes numerous advantages in terms of convenience, effectiveness, suitability, and patient compliance. The mucociliary clearance stands as a physiological factor mainly involved in decreasing of the drug's time in the nasal environment. The quick clearance of the drug from the nasal cavity due to this self-clearing process, which reduces the time required for the medicine to reach the systemic bloodstream or the central nervous system [7].

Therefore an in situ gelling strategy is needed to prevent drugs from rapidly draining from the nasal cavity when administered as simple aqueous solutions and to extend their stay in the nasal cavity. Such low viscosity polymeric solutions are easily delivered, ensuring optimal nasal deposition, and transform into gels when they come into touch with the nasal mucosa. The sol-gel change encouraged by temperature change. The in vivo formation of a polymeric network and mucoadhesive nature of polymers prolongs the interaction time amid the drug and the site of action/absorption and also guarantees a sustained release of pharmaceutical ingredients [8].

Bearing in mind the pharmacokinetic properties of Atomoxetine HCl , the intranasal drug delivery system could represent a new and valid pharmaceutical form in treatment of ADHD. Present investigation deals with the development of a thermosensitive, mucoadhesive in-situ gel for intranasal delivery of Atomoxetine HCl to achieve rapid onset of action and increase its therapeutic effectiveness for a longer period thus reducing dose frequency and side effect.

2. MATERIALS AND METHODS

2.1. Drugs and chemicals

Atomoxetine HCl was gifted by Aarti Chemicals Mumbai, India. Poloxamer 407 was purchased from Yarrow Chem Products Pvt. Ltd., Mumbai, India. Dialysis membrane (12,000–14,000 M W.) was purchased from HiMedia Laboratories Pvt. Ltd. Mumbai, India. HPMC K4M, HPMC K100 and Carbapol was obtained from Loba Chemie Pvt. Ltd, Mumbai, India. All other chemicals and reagents of analytical grade were used.

2.2. Selection of Poloxamer 407 concentration:

The concentrations of Poloxamer 407 plain and with containing Atomoxetine HCl varying from 15% w/v to 22% w/v were prepared preliminarily to determine the lowest possible concentration showing thermo-reversibility below 34 °C (physiological temperature of nasal cavity). Gelation temperature was determined by using visual inspection method [9]. 10 mL formulation was taken in a beaker and placed on magnetic stirrer. Formulations were stirred with magnetic bead at slow speed. A temperature of apparatus was increased gradually (1 °C/ min.) The formulation were observed for gelation throughout the experiment. The temperature value at which the magnetic bead stops spinning was taken as gelation temperature [10].

2.2. Optimization of in situ gel of Atomoxetine by experimental design

The optimal design was applied using Design-Expert 13.0.5.0 software to study the effect of independent variables (concentrations of mucoadhesive polymers) on physicochemical properties of in situ gel of Atomoxetine. These factors and their levels are illustrated in Table 1.

Table 1: Levels of Independent variables

Sr. no	Autonomous variables	Levels		
		Minimal (-1)	Intermediate (0)	Higher (1)
1.	HPMC K4M	50	75	100
2.	HPMC K100	50	75	100
3.	Carbopol	50	75	100

Also, the effects of these variables were investigated on the gelation temperature, mucoadhesive strength and % CDR. The optimum formulation was selected regarding gelation temperature at physiological temperature range, the minimum %CDR at 7^{th} hr and maximum range of mucoadhesive stregth. Furthermore, the percentage of error between the expected and the observed results were calculated. Finally, the optimized formulation was selected for further investigation.

2.3. Preparation of in situ gels:

The Poloxamer gel was prepare by using cold method. Poloxamer solutions were prepared by dissolving precisely weighed quantities of Poloxamer 407 (18%) in the cold distilled water at 5 ^{0}C and stirred at 500 rpm for 1 hr to avoid forth formation. Temperature was maintained throughout the procedure. The cloudy solutions were kept at refrigerated conditions overnight. Mucoadhesive polymer were separately dissolved in cold distilled water at 5 ^{0}C. Poloxamer solutions were then added to solutions of mucoadhesive polymer with constant stirring for 1 hr. The Atomoxetine HCl was separately dissolved was added in above solution with stirring for 45min at 300 rpm. The final volume of formulation was adjusted with distilled water up to 100% and the prepared gel was kept at 4 °C [11].

Table 2: Compositions of studied in situ formulations

Sr.no.	Batch code	Drug (mg)	Poloxamer 407 (%)	HPMC K4M (%)	HPMC K100 (%)	Carbopol (%)
1	F1	250	18%	0.2%	-	-
2	F2	250	18%	0.3%	-	-
3	F3	250	18%	0.4%	-	-
4	F4	250	18%	-	0.2%	-
5	F5	250	18%	-	0.3%	-
6	F6	250	18%	-	0.4%	-
7	F7	250	18%	-	-	0.2%
8	F8	250	18%	-	-	0.3%
9	F9	250	18%	-	-	0.4%

2.4. Evaluation of gel:

The resultant formulations were studied for various evaluation parameters.

2.4.1. Determination of appearance and clarity:

The formulations were examined for appearance and clarity by observation next to black and white background to check the existence of any foreign matter.

2.4.2. pH Determination:

The formulations evaluated for pH by using digital pH meter, which was pre calibrated using standard pH 4 and 7 buffers [12].

2.4.3. Gelation temperature:

Gelation temperature was determined by using visual inspection method. 10 mL formulation was taken in a beaker and placed on magnetic stirrer. Formulations were stirred with magnetic bead at slow speed. A temperature of apparatus was increased gradually (1 °C/ min.) The formulation were observed for gelation throughout the experiment. The temperature value at which the magnetic bead stops spinning was taken as gelation temperature [13].

2.4.4. Drug content:

Gel equivalent to 10 mg of Atomoxetine HCl was diluted with phosphate buffer of pH 6.4. After suitable dilutions, the drug concentration was determined at 269 nm using UV visible spectrophotometer and the drug concentration was calculated using the formula [14].

$$\% \text{ Drug Content} = \frac{\text{Concentration of drug in the sample solution} \times 100}{\text{Equivalent concentration of drug taken}}$$

2.4.5. Determination of gelling capacity:

Based on formulation behaviours such as gelation & erosion time of formed gel owing to environmental changes, gelling capacity was calculated [15].

+- Gelled after few minutes and dissolves rapidly (with in mins),
++- Gelled after few minutes and remains intact for few hours,
+++- Gelled immediately and remains intact for extended period of time

2.4.6. Viscosity of formulation:

Viscosity was observed at solution state and after gelation using Viscolead viscometer (Fungilab) with spindle number L3 at 50 rpm. [16].

2.4.7. Gels strength:

An accurately weighed quantity (10 g) of preparation was taken in a 25 ml measuring cylinder and was converted to gel form. A weight of 5 g was placed on to the gel, the time required for weight to deep 5 cm through gel was measured [17].

2.4.8. Mucoadhesion Strength:

The mucoadhesive potential was computed by calculating the force needed for split-up of formulation from sheep nasal mucosal tissue by utilizing modified pan balance for each formulation [16]. A sheep nasal tissue was acquired from slaughter-house within 30 min after the animal was sacrificed. Nasal mucosal membrane was carefully removed from its nasal cavity and was stored in a normal saline solution. Blood & cartilage from the mucosal membrane was removed. The mucosal side of membrane was immediately tied to each glass vial. These vials were conditioned at 34 ^{0} C before use. One vial was fixed on right arm of a height-adjustable balance in inverted position whereas another vial was positioned below the first vial. Specified amount of gels were applied onto the mucosa and both vials were permitted to come in close contact by adjusting the height of the inverted vial. To establish an intimate contact between tissue and the sample was vials were allowed in close contact for 2 min. A dummy granules were used as weights and were gradually added in the pan on other side. The lowest weight needed for separation of two membranes i.e. detachment stress is expressed as the mucoadhesive strength and calculated using the equation [28].

$$\text{Detachment stress (dynes/cm)} = \frac{mg}{A}$$

Where,
m = Lowest weight needed for separation of two vials in grams
g = Acceleration due to gravity (980 cm/s$^{2)}$ and
A = Area of membrane exposed.

2.4.9. In vitro drug release studies.

In vitro diffusion studies from the gel were done with Franz diffusion cell through dialysis membrane dipped in phosphate buffer pH 6.4 for 24 h before use. The dialysis membrane was positioned in middle of donor and receptor cells. The gel equivalent to 10 mg Atomoxetine HCl was applied on the donor compartment in a

homogeneous manner. The receptor compartment was filled with 25 mL phosphate buffer pH 6.4. The whole cell was maintained at 34±1°C and stirred constantly at 100 rpm. At scheduled time intervals, Aliquots of 1 mL sample were collected from receptor compartment and replenishment with an equal volume of buffer for 6 hrs. The samples after appropriate dilutions were analysed at λ max of 269 using spectrophotometer [19].

2.4.10. Optimization of batch:

The data obtained from gelation temperature, mucoadhesive strength and % CDR were used to find the optimized batch. This optimized batch was further subjected to ex vivo drug release and release kinetics, IR, characterisation.

2.4.10. Fourier Transformation Infrared Spectroscopy (FTIR):

The structural features of pure drug and optimized formula were estimated by FTIR. The spectra were recorded for using Bruker ATR-IR spectrophotometer. The sample is directly placed on clean ATR crystal and hold in probe. The sample were scanned over the range of 4000-450cm-1 [20].

2.4.12. Differential scanning Calorimetry (DSC):

The thermal stability of pure drug and optimized formula were determined by DSC. The DSC thermograms were recorded by using. Differential scanning Calorimeter (Universal V4.5A TA Instrument ((SDT Q600 V20.9 Build 20)). Sample were placed in aluminium pan and heated in the range 0-350 ^{0}C at the rate of 1^{0}C/min. in Nitrogen atmosphere [21].

2.4.13. Ex vivo permeation studies using sheep nasal mucosa:

For the best formulations, an ex vivo drug permeation investigation was conducted. A Fresh sheep nasal mucosa was positioned in middle of donor & receptor cells, and the same procedure was followed as in vitro drug release [19].

2.4.14. Drug release kinetics:

Mechanism of release from in situ gel was understood by fitting the diffusion data of optimized formulations in model dependent kinetics. Based on the slope and the regression coefficient values (r^2) obtained from the above models, the mechanism of release was determined.

3. RESULT AND DISCUSSION

3.1. Optimization of Concentration of Poloxamer 407:

Table 3: Optimization of Concentration of Poloxamer 407.

Formulation Batch	**Concentration of poloxamer** (%w/v)	**Atomoxetine HCl** (mg)	**Gelation Temperature** (°C)
G1	15	-	Above 45
G2	16	-	40.8
G3	17	-	35.9
G4	18	-	32.9
G5	19	-	28.7
G6	20	-	24.5
G7	21	-	23.8
G8	22	-	21.2
G9	15	250	Above 45
G10	16	250	41.7
G11	17	250	37.3
G12	18	250	33.6
G13	19	250	29.1
G14	20	250	26.4
G15	21	250	24.7
G16	22	250	23.4

From results, it was observed that only 18% of gel with drug (G8) showed ability to form gel in temperature range of 32°C to 34°C. So, 18% (w/v) concentration was used for further studies (Table 3). Furthermore, it was observed that Poloxamer 407 concentration and time required for the formation of gel and gelation temperature are inversely proportional (G1 to G8). When Atomoxetine was added, it was found that gelation temperature of formulations (G9 to G16) increased remarkably for each concentration of gelling agent; however, the pattern was similar. The reduced gelation temperature associated with higher Poloxamer 407 concentrations could be owing to the higher quantity and volume of micelles present at low temperatures. The gel structure gets more closely packed with the arrangement in the lattice pattern as the concentration of Poloxamer 407 increases, and gelling happens quickly at low temperatures. The gelation temperature is increases when the Atomoxetine is included into in situ gels. This could be due to Atomoxetine's water soluble nature, which could alter the micellar association process of Poloxamer 407 gels, causing them to gel at a higher temperature.

3.2. Evaluation of gel

Table 4: Appearance, pH, Gelation Temperature, Gelation Time and Gelling Capacity of formulations.

Sr.no	Formulation	Appearance	pH	Gelation Temperature	Gelation Time	Gelling capacity
1	F1	Transparent and Clear	6.5	35.2	2.56	***
2	F2	Transparent and Clear	6.1	34.2	2.32	***
3	F3	Transparent and Clear	6	32.4	2.21	**
4	F4	Transparent and Clear	6.3	37.4	3.34	**
5	F5	Transparent and Clear	6.1	36.9	3.15	**
6	F6	Transparent and Clear	5.7	37	3.33	*
7	F7	Cloudy and opaque	5.8	40.4	4.22	**
8	F8	Cloudy and opaque	5.9	40.2	4.22	*
9	F9	Cloudy and opaque	5.5	39.6	3.54	*

3.2.1. Determination of visual appearance and clarity:

The formulations were examined for visual appearance and clarity by visual observation against a white and black background to check the presence of any particulate matter. Gel base was liquid at 4°C and formed gels at the body temperatures. Results shows that the formulation from F1 to F6 containing HPMC were transparent and clear & the formulation from F7 to F9 containing Carbopol were cloudy opaque (Table 4).

3.2.2. Determination pH of gel:

The formulated gels were evaluated for pH using calibrated digital pH meter at room temperature. Despite its normal physiological pH range of 4.5 to 6.5, the nasal mucosa may withstand pH between 3 and 10. To ensure compatibility with nasal mucosa, the formulations should be in the range of nasal pH (5.5–6.5). All gel formulations showed pH around that of the nasal mucosa, ranging from 5.5 to 6.5, (Table 4) indicating that they were non-irritating. The concentration of mucoadhesive polymers in the formulations was responsible for the pH variations. Among all the formulations, pH of Carbopol formulation was less, as carbopol is a homo and copolymer of acrylic acid which is cross-linked with polyalkenyl polyether making it more acidic. Lysozyme, which is generated in nasal secretions and is responsible for killing some microorganisms at acidic pH, is inactive at alkaline pH, leaving nasal tissue vulnerable to microbial infection. As a result, it's best to keep the pH of your mixture between 4.5 and 6.5.

3.2.3. Determination of gelation temperature:

Gelation temperature all the formulations was observed in a range of 32.4 to 39.6 ^{0} C (Table 4). The temperature of the nasal mucosa has been recorded 32 to 34 ^{0} C, which is lower than the typical body temperature. As a result, an in-situ gel developed should be liquid at room temperature but quickly transforms into a gel when it comes into contact with the nasal mucosa i.e. at 32-34 ^{0} C. According to the findings, as the concentration of Poloxamer increases, the gelation temperature decreases. The decreased gelation temperature

was result of the interaction between the hydrophobic component of polymer molecule, which could disrupt micelle structure and enhance entanglement of micelle. Increase in temperature promoted the micelles formation due to the negative coefficient of solubility of micelles. Addition of mucoadhesive agent has shown to influence the sol-gel transitions of gel, where gelation temperature lowering effect might be due to increased viscosity after dissolution of mucoadhesive polymer.

3.2.4. Gelation time:

All the formulations took between 2.56 and 3.54 minutes to convert from sol to gel (Table 4). Formulations must be converted to gel form below 35 °C to avoid loss; otherwise, it would remain in a liquid condition at body temperature and will easily drain out when administered in the nasal cavity. With the increase in gelation temperature, the gelation time for all formulations increased. It supports the reason that lower the gelation time faster is the gelation.

3.2.5. Gelling capacity

Formulations F6, F8, and F9 gelled and dissolved rapidly after a few minutes, whereas F3 to F7 converted to gel after a few minutes and stayed intact for a few hours. Formulations F1 to F2 gelled faster and stayed intact for a longer amount of time. (Table 4)

Variations in gelling capacity were the results of different concentrations and type of mucoadhesive polymers. Formulations containing HPMC K4M showed maximum gelling capacity as compared to formulations containing HPMC K100 and Carbopol.

Table 5: Gelling strength, Viscosity, Drug content, Mucoadhesive strength and %CDR of formulations.

Sr.no	Formulation	Gelling strength	Viscosity	Drug content	Mucoadhesive strength. (dynes/cm2)	%CDR After 7 hrs
1	F1	2.4	92.93	96.8	1321.67	97.4058
2	F2	2.2	146.27	96.07	1721.32	94.31884
3	F3	2.9	89.13	96.47	2119.86	92.46377
4	F4	3.46	89.13	96.9	1541.29	98.76812
5	F5	3.51	121.91	98.33	1623.73	97.55072
6	F6	3.57	136.91	97.3	1976.11	93.75362
7	F7	4.28	111.61	97.31	1721.59	99.058
8	F8	4.32	158.56	97.33	1998.23	97.52174
9	F9	4.45	189.52	98.34	2419.43	92.3913

3.2.6. Gelling strength

The formulations F1, F2, F3 showed lesser strength than F4, F5, F6 followed by F6, F7, F8 formulations (Table 5). From the obtained results it was observed that the gelling strength improved when the gelling agent concentration was increased. This is because of micellar nature of poloxamers made up of cubic oriented micellar subunits and excessive hydrogen bonding between water molecules and the polymer's ethereal oxygen results in the creation of tight bonds.. The gel structure gets tightly packed with the arrangement in the lattice pattern as the polymer concentration increases. The mucoadhesive agent added also showed the effect on gel strength of formulations. It was found that, greater the percentage of mucoadhesive agent, greater was the gel strength seen.

3.2.7. Determination of viscosity.

From the results obtained a direct correlation was found between viscosity and concentrations of the gelling agent. Formulations F1, F4, F7 showed lesser viscosity than F2, F5, F8, followed by F3, F6, F9, and there was an increases in viscosity with an increase in temperature, but variation seen due to combination of polymers (Table 5). This indicated the formation of temperature induced gel structure of Poloxamer 407. All the formulations remained liquid up to certain temperature and with an increase in temperature there was a rise in the viscosity which proves the formation of gel. This confirms that formulations change in their behavior from liquid-like (Newtonian) to gel like (non-Newtonian) when the temperature increases. The variations in viscosities were seen due to a sudden rise in micellar concentration at higher temperatures .In addition to this, the mucoadhesive agent showed similar viscosity enhancing effect with an increase in its concentration. Among all, the formulations with Carbopol showed higher viscosity.

The dynamic viscosity of all the formulation was measured as the change in RPM under a physiological condition. The viscosity of the formulation decreased as the RPM increased, which showed the character of pseudoplastic fluid (Figure 1). The pseuodoplastic property of Poloxamer 407 formulation under physiological condition is in favour of controlled drainage of drug from the nasal cavity.

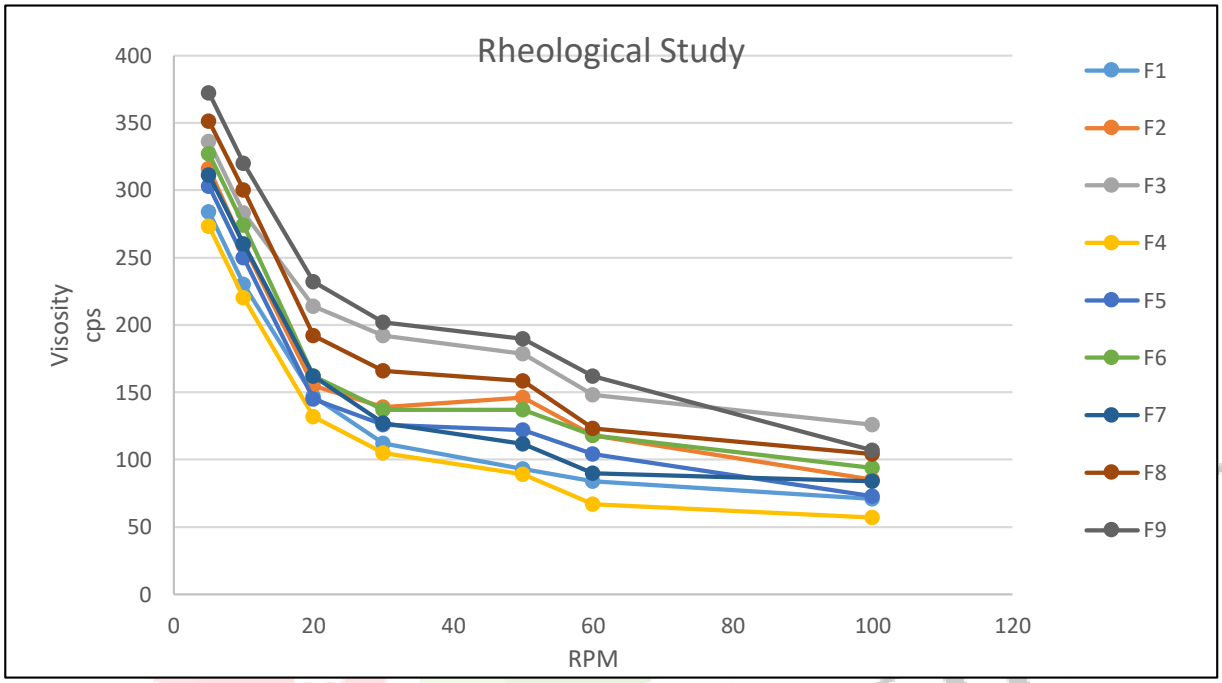


Figure 1: Rheological studies of formulations.

3.2.8. Drug content

The drug content was found to be in acceptable range for all the formulations. Percent drug content of formulations was seen to be between ranges of 96.07 to 98.34 (Table 5). F7 and F9 formulations containing Carbopol showed maximum drug content which indicated the efficient loading and uniform distribution of drug throughout the gel.

3.2.9. Mucoadhesive strength

The mucoadhesive strength of all the formulations were observed to be in a range of 1321.67 to 2419.43 dyne/ cm^2 (Table 5) . Both Poloxamer-407 and mucoadhesive agent showed an effect on mucoadhesive strength of gels. The concentration of both polymers was directly proportional to mucoadhesive strength. Highest mucoadhesive strength was shown by the formulations containing carbopol 934P, followed by the formulations with HPMC K4M, and HPMC K100. Reason was attributed to, carbopol has a very high percentage (58%-68%) of carboxylic groups that undergoes hydrogen bonding with sugar residues in oligosaccharide chains of the mucus membrane, which results in the creation of a toughened network between polymer and mucus membrane, owing to strong interaction between hydrogen bonding groups of carbopol and mucin glycoproteins. In addition, carbopol also adopts favorable macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. On the other hand, as concentration of HPMC increases, mucoadhesion also increased. This was due to wetting and swelling of HPMC, intimate contact, interpenetration of HPMC chains with mucin molecules resulting in entanglement and formation of

weak chemical bonds. Thus, it was concluded that higher the mucoadhesive strength, prolongs the retention time which leads to enhanced absorption across mucosal tissues.

3.2.10. In vitro diffusion studies.

Formulations F1, F2 and F3 exhibited 97.4058, 94.31884 and 92.46377% of drug diffusion, respectively. Formulations F4, F5 and F6 exhibited 98.76812, 97.55072 and 93.75362 % of drug diffusion, respectively. Formulations F7, F8 and F9 exhibited 99.058, 97.52174 and 92.3913% of drug diffusion in, respectively in 7 hrs (Table 5) From the above results, it was concluded that as the concentration of polymers increased, there was a decrease in the drug release rate seen (Figure 2). Increase in concentration of polymer causes increase in viscosity of gel layer with longer diffusional path and this could cause reduction in the drug release which may be due to reduction in the water channel number. Formulations with higher HPMC grade have slower drug release when compared to the formulations with HPMC lower viscosity grade, formulation containing Carbopol 934P showed faster drug release because of its anionic nature and are reported to demonstrate permeation enhancing properties. Presence of Carbopol resulted in very rapid dissolution and release of highly soluble drug due to its rapid swelling and dissolution, and also these resulted in increased concentration of ionized carboxyl group to a level that was required to cause conformational changes in the polymer chain. Electrostatic repulsion of the ionized carboxyl group results in decoiling of the polymer chain, resulting in the relaxation of the polymer network. Apart, these polymers were also known to express a high Ca^{2+} binding ability, and also in an increase inter accessibility of Ca^{2+} binding sites owing to relaxation of the polymer network, causing the drug to release more. Higher HPMC grade higher is the viscosity and thus slower is the drug release. The amount of drug release was found to be in the order of carbopol 934P> HPMC K00> HPMC K4M.

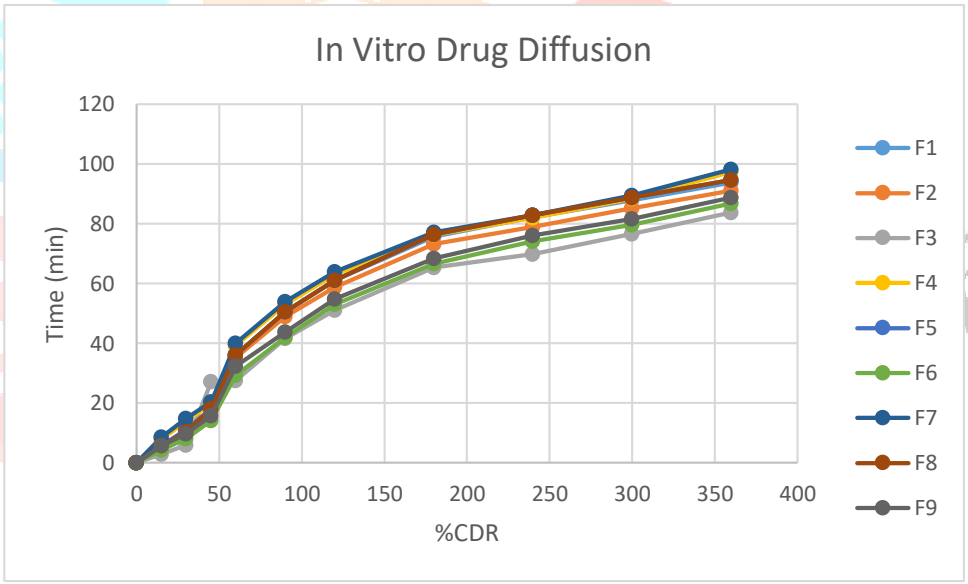


Figure 2: In vitro drug diffusion profile

3.3. Effect of variables:

The statistical analysis of all data obtained from experiment is done by Design-Expert 13 software. The optimum formula was generated after applying specific constraints on gelation temperature, mucoadhesive strength and in vitro drug diffusion. After which the obtained optimum formula was prepared and studied for gelation temperature, mucoadhesive strength, in vitro drug diffusion and further characterization such as ex vivo drug diffusion.

For the design study four independent variables and three response variables, Poloxamer 407 (A), HPMC K4M concentration (B), HPMC K100 (C) and Carbopol concentrations (D) as an independent variables where Gelation Time (Y1), Mucoadhesive Strength (Y2); and Drug diffusion (Y3) as response variables. These responses were fitted individually to Linear for all responses using linear regression to obtain the model of choice with the highest adjusted and predicted r^2. The analysis of variance (ANOVA) is performed to identify the significant factor, better fit and the best formulation possible. Value of "Prob > F" less than 0.05 indicate model terms are significant. Value greater than 0.1 indicate the model terms are not significant. The optimum

formulation was selected considering gelation temperature in range of 32-34 ^{0}C, maximum range of mucoadhesive strength and minimum drug diffusion at 7 hr. The desirability index was used in the data optimization of D-optimal design. Furthermore, the percentage of error between the expected and the observed results were calculated. Finally, the optimized formulation was selected for further investigation.

Table 6: Effects of variables

	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2	Response 3
Run	A:Poloxamer 407 %	B:HPMC K4M %	C:HPMC K100 %	D:Carbopol %	Gelation Temp ^{0}C	Mucoadhesive strength dyne/cm square	In Vitro DR %
1	18	0.2	0	0	34.2	1321	97.4058
2	18	0.3	0	0	32.8	1721	94.31884
3	18	0.4	0	0	32.2	2119	92.46377
4	18	0	0.2	0	35.8	1541	98.76812
5	18	0	0.3	0	34.9	1623	97.55072
6	18	0	0.4	0	33.4	1976	93.75362
7	18	0	0	0.2	39.4	1721	99.058
8	18	0	0	0.3	38.2	1998	97.52174
9	18	0	0	0.4	37.7	2419	92.3913

Fit Summary:

Table 7: Fit summary of responses.

Response	Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Particle size	Linear	0.0009		0.9152	0.7895	Suggested
Mucoadhesive strength	Linear	0.0016		0.9073	0.8165	Suggested
%CDR	Linear	0.0026		0.8873	0.7782	Suggested

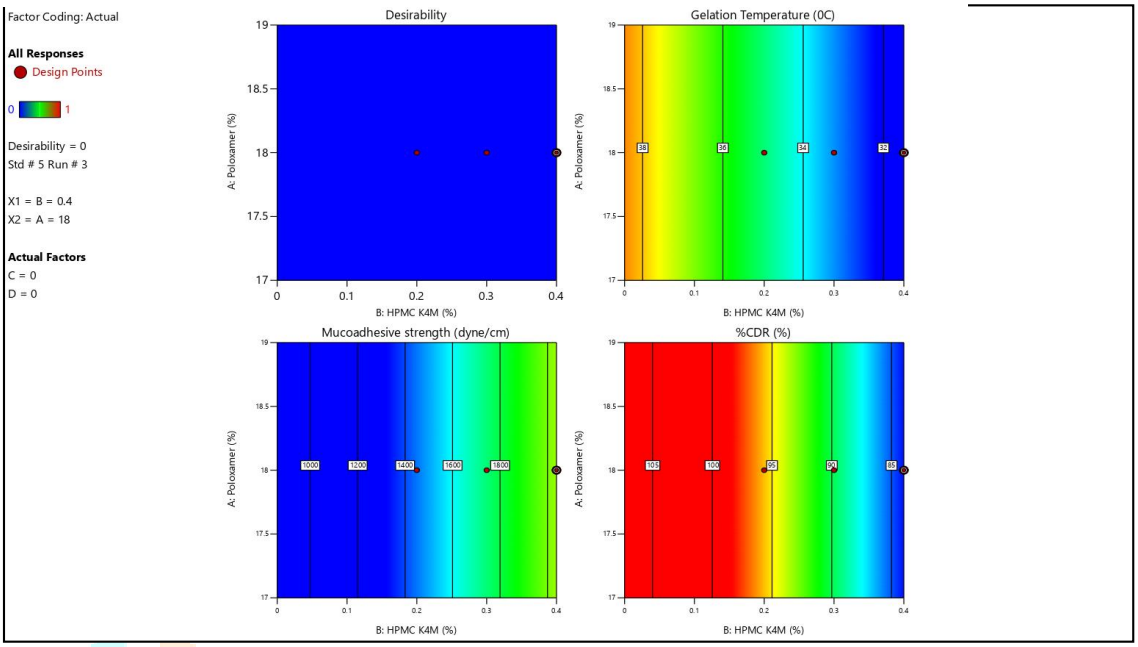
3.4. Optimization of Batch:

By using the data of gelation temperature, mucoadhesive strength and % drug diffusion optimized batch was found to be as follows (Table 8).

Table 8: Composition of optimized batch and predicted results.

No.	Poloxamer 407	HPMC K4M	HPMC K100	Carbopol	Gelation Temperature	Mucoadhesive strength	%CDR	Desirability
1	18.000	0.138	0.145	0.146	34.157	2243.884	85.543	0.444

Figure 3: Responses of optimized batch for HPMC K4M



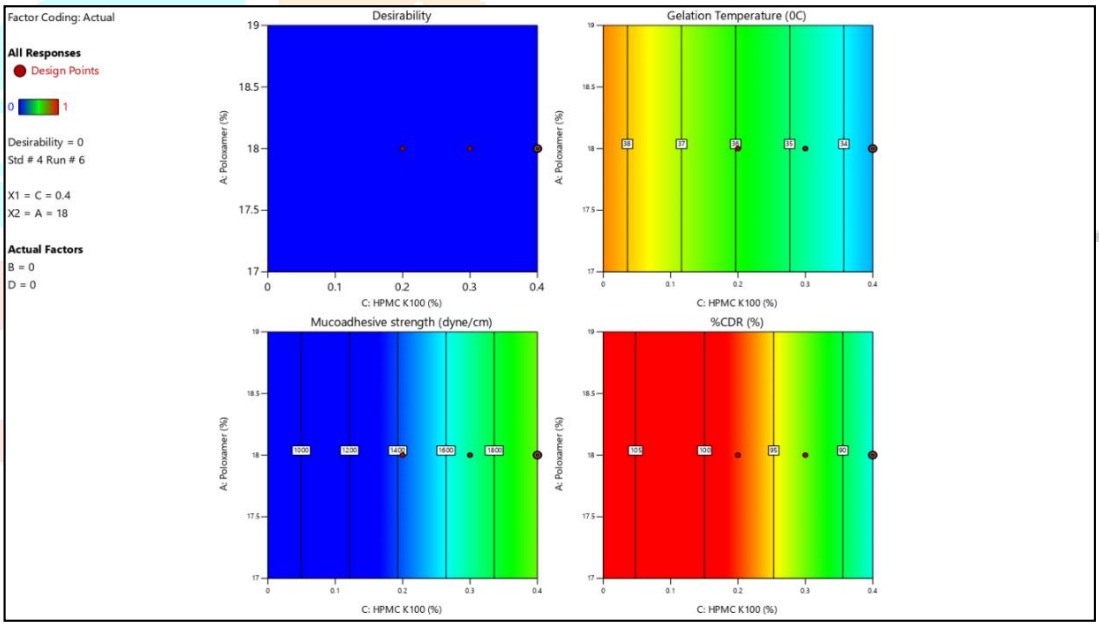


Figure 4: Responses of optimized batch for HPMC K 100

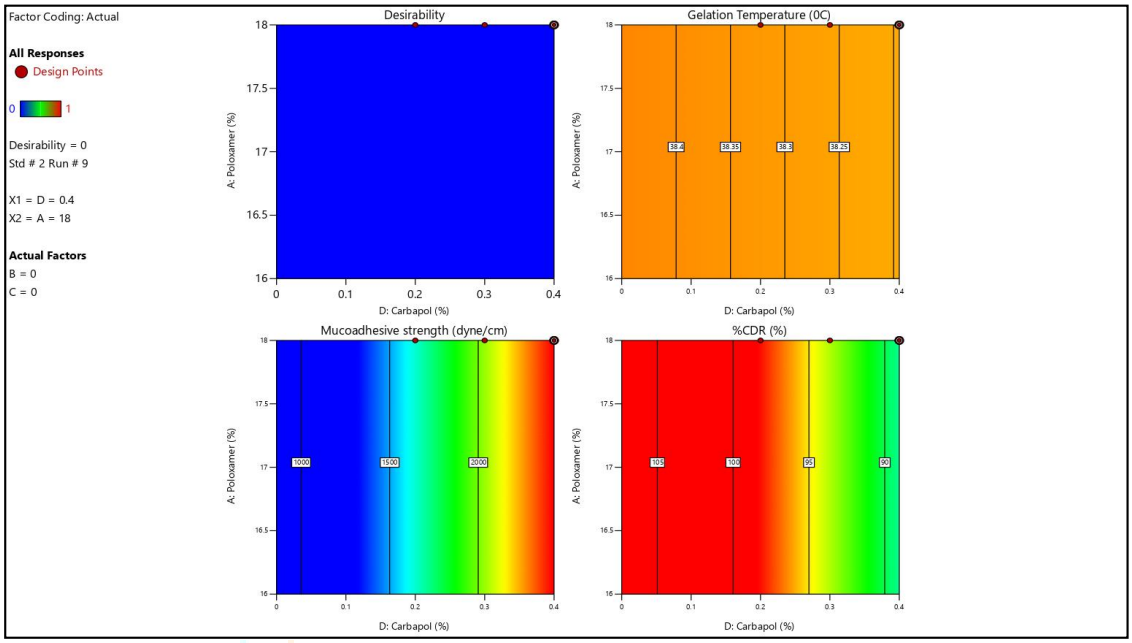


Figure 5: Responses of optimized batch for Carbopol

Table 9: Observed responses of optimized batch

Response	Predicted	Observed	Residual
Gelation Temperature	34.1573	32.35	1.80
Mucoadhesive strength	2243.88	1826.56	417.32
%CDR	85.5427	92.0467	-6.5

3.5. Characterization of optimized formulation:

3.5.1. Fourier Transformation Infrared Spectroscopy (FTIR):

The structural features of pure drug and optimized formula were estimated by FTIR.

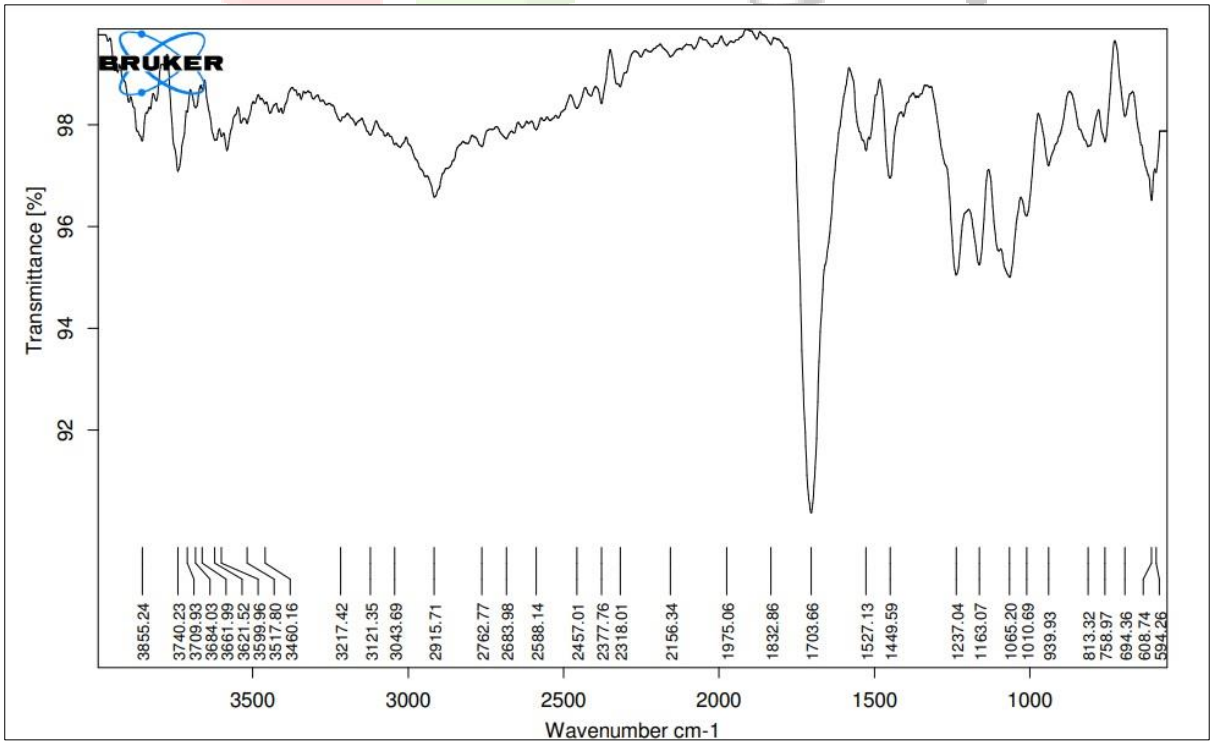


Figure 6: FTIR spectra of optimized formulation

Table 10: Spectrum interpretation of optimized batch.

Group	Wave number(cm-1)	
	Pure drug	Formulation
C-H stretching	2691.33	2915.71
N-H bending	1593.60	1703.66
C-H bending	1449.42	1449.59
C-O stretching	1235.82	1237.04
C-O stretching	-	1065.20
R-NH stretching	1004.56	1010.69
C-H bending	752.65	758.97
C-H bending	689.78	608.74

Pure drugs shows characteristic peaks as mentioned in table 10. These peaks have appeared in in situ gel formulation which indicates that there was no chemical interaction takes place between drug and excipients.

3.5.2. Differential scanning Calorimetry (DSC):

The thermal stability of optimized formula were determined by DSC. The DSC thermograms were recorded by using Differential scanning Calorimeter (Universal V4.5A TA Instrument (SDT Q600 V20.9 Build 20). The DSC thermogram of optimized formulation is found to be as fig.7.

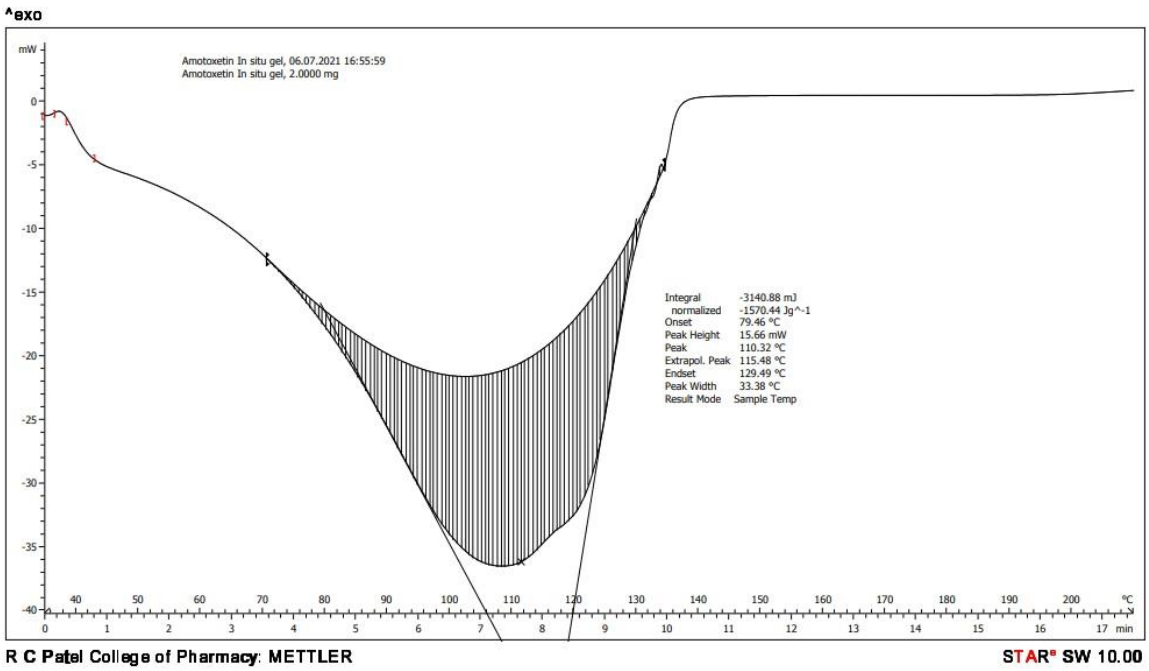


Figure 7: DSC Thermogram of optimized formulation

The thermogram of optimized formulation shows the broad endothermic peak which was near to the actual melting point of Simvastatin that i.e., 165^0C reveals that Atomoxetime HCl formulations are stable.

3.5.3. Ex vivo permeation studies:

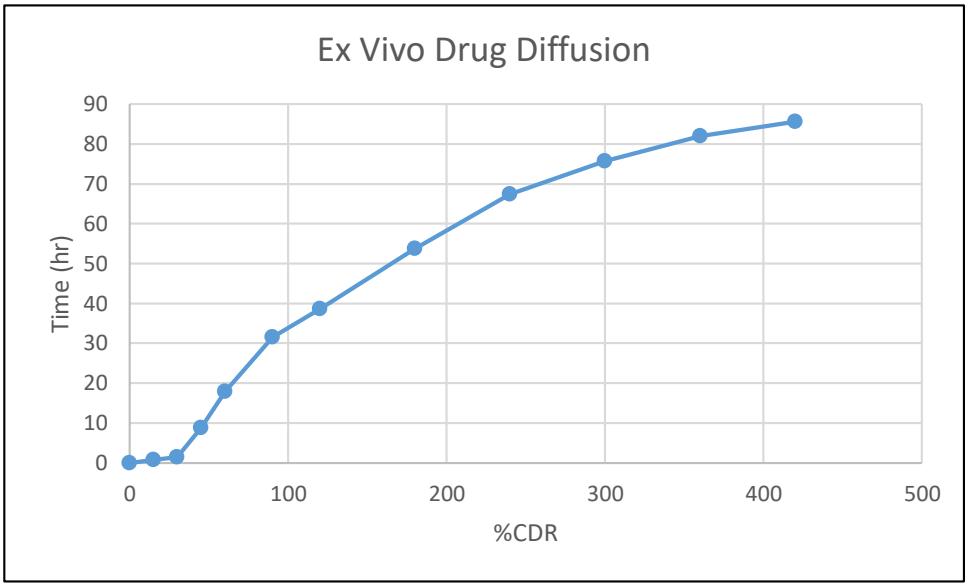


Figure 8: Ex vivo drug diffusion profile of optimized formulation

Studies were performed to know the amount of drug that has actually diffused across the tissue over the period of time. It was observed that formulations exhibited drug diffusion up to 84.54 from nasal mucosa in 7 hrs and up to 96.37 in 24 hrs (figure 8). When compared to dialysis membrane, drug diffusion from mucus membrane was less and the reason was attributed to high thickness of nasal mucosa. The ex vivo drug diffusion conditions may be different from those likely to be encountered in the nasal cavity. However, the results clearly show that the gels have the ability to retain drug for prolonged period of time (7 hour) and that premature drug diffusion will not occur.

3.5.4. Drug release kinetics:

Mechanism of release from in situ gel was understood by fitting the diffusion data of optimized formulations in model dependent kinetics. Based on the slope and the regression coefficient values (r^2) obtained from the above models, the mechanism of release was determined.

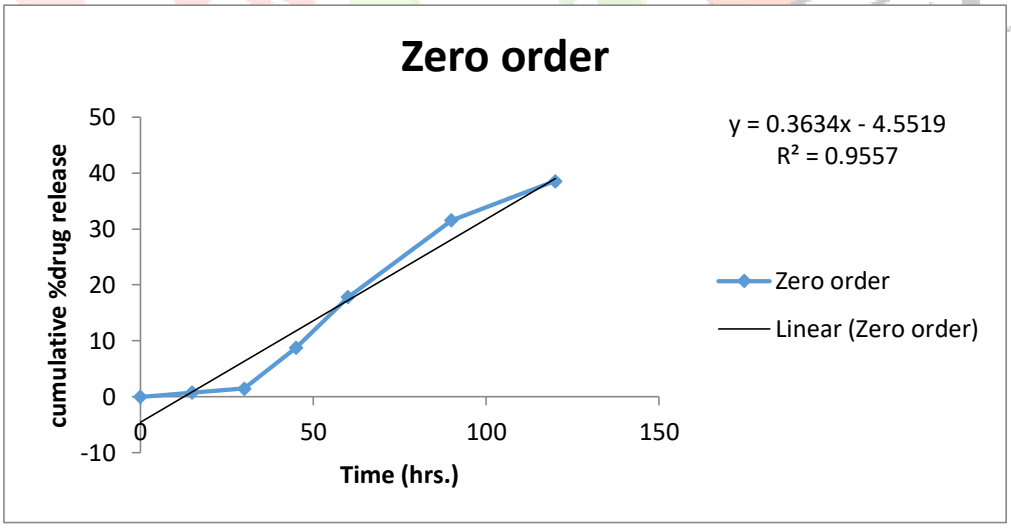


Figure 9: Zero order release model of optimized formulation

Table 11: Ex vivo release kinetics of optimized batch.

Release model	Equation	R^2
Zero order	$C_t = C_0 + K_0t$	0.9557
First order	Log C = Log C_0 + K_t/2.303	0.9519
Higuchi model	Q = KH √t	0.778
Hixon-Crowell model	$C_0^{1/3}$-$C_t^{1/3}$=K_{HC}t	0.9539
Korsemeyer – peppa model	$M_t/M\infty = Kt^n$	0.5242

A linear form of various kinetic models was plotted based on drug release data for realizing the mechanism of drug release. Some of the most important release kinetic models are as follows: zero-order (cumulative percent drug release vs time), first-order (log cumulative percent drug remaining vs. time), Higuchi (cumulative percent drug release vs. square root of time), Hixon crowells (cube root of cumulative drug release vs. time) and Korsmeyer Peppas (log percent cumulative drug release vs. log time). The R^2 calculated for the linear curve was obtained by regression analysis to determine the release kinetics of the optimum formulation. A kinetic model with a regression coefficient near to one; is a desirable model for the release profile of that formulation. Here, the drug release mechanism for optimum formulation can be best fitted by the zero order model.

4. CONCLUSION

From the present attempt, it was concluded that the in situ gel formulation proved to be efficient carrier for improved delivery of Atomoxetine HCl. Atomoxetine HCl can be successfully incorporated within in situ gel with high efficiency by using cold method. In situ gel was found to be a promising approach to sustain the drug release for an extended period of time in nasal cavity. In situ gel have been anticipated to provide stability as compared to conventional dosage form such as drops and gels in terms of retention time, leakage from nasal cavity, etc. The future scopes for the work are in vivo studies. These results put together convincingly prove that in situ gel of Atomoxetine HCl can be a safe and promising alternative to conventional delivery systems displaying superior drug release.

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