Design and Optimization of Thermo-reversible Nasal *in situ* Gel of Atomoxetine Hydrochloride Using Taguchi Orthogonal Array Design

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ABSTRACT: The present investigation was aimed to develop a thermo-reversible nasal *in situ* gel of atomoxetine hydrochloride (AH) with reduced nasal muco-ciliary clearance in order to improve residence time and targeting the brain through nasal mucosa for the treatment of attention-deficit hyperactivity disorder (ADHD). *In situ* gel formulations were prepared using different concentrations of the thermo-gelling poloxamer 407 and mucoadhesive polymers. Temperature-triggered ionic gelation is the mechanism involved. Taguchi L9 OA experimental design was employed for the optimization of the effect of independent variables (Poloxamer 407 and Carbopol 934P) on the response (gelation temperature). *In situ* gel formulation F4 having 20% poloxamer 407 and 0.3% carbopol 934P and formulation F6 having 20% poloxamer 407 and 0.2% HPMC K100 were optimized based on evaluation parameters. The gelation temperature of F4 and F6 was found to be 37°C ± 0.4 and 37°C ± 0.2, drug content 98.34 and 98.33% and drug release was 83.18, 82.4% in 4 hrs with a flux of 436.9 and 428.1 µg.cm^2/hr, respectively. The release pattern of drug followed first-order kinetics with Higuchi release mechanism. The value of 'n' from Korsemeyer equation indicated the anomalous diffusional drug release. This study concluded that in situ gel enhanced the nasal residence time and thus may improve the bioavailability of the drug through nasal route by avoiding first pass metabolism.

Key words: *In situ* gel, thermo-reversible, poloxamer-407, carbopol-934P, korsemeyer, nasal residence time, bioavailability.

INTRODUCTION

Intranasal administration is an approach for rapid-onset delivery of medications and to circumvent their first-pass elimination when taken orally. Nasal administration of drug offers various advantages like the rapid onset of action by fast absorption, higher bioavailability allowing lower doses, avoidance of liver or gastrointestinal metabolism, gastrointestinal irritation, and enhanced patient compliance by self-medication.[1] Atomoxetine HCl is the first non-stimulant drug approved for the treatment of attention-deficit hyperactivity disorder (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. Patients who cannot absorb orally administered drugs, unconscious patients etc., cannot use the conventional oral dosage forms. One modern way to cope with these difficulties is the usage of novel technology like *in situ* nasal gelling system. The *in situ* gel is referred to a kind of preparation in a form of solution condition, and with the changes in the physiological environment of administration site, the phase changes and forms a gelatinous semi-solid preparation. However, usage of nasal drug delivery system is limited due to the muco-ciliary clearance which severely limits the residence time for the drug to be absorbed. Hence this problem can be overcome with the design of mucoadhesive preparation of the drug which will increase contact time between the

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formulation and mucosal layers of nasal cavities, thereby enhancing drug absorption.[2] The main principle involved in the formation of in situ gel is the temperature-induced gelation. Gelling of the solution is triggered by the change in temperature, and sustained drug delivery can be achieved by the use of a polymer that changes from solution to gel at the physiological temperature. Due to the unique property of thermo-reversible polymers that are characterized by changing their conformation, solubility and hydrophilic/hydrophobic balance as a response to changes in the temperature and also by having a lower critical solution temperature (LCST).[3] Below this temperature, the polymer chains are water soluble and form hydrogen bonds with the water molecules. However, as the temperature is raised above the LCST, the hydrogen bonds are broken and the hydrophobic associations dominate, causing the polymer in dilute solution to undergo coil-to-globule phase transitions, resulting in macroscopic phase separation and polymer aggregation. The mechanisms underlying the direct nose to brain drug delivery is intracellular and extracellular transport mediated routes. The intracellular transport mediated route is a relatively slow process, taking hours for intra-nasally administered substances to reach the olfactory bulb. The extracellular transport mediated routes could underlie the rapid entrance of drug into the brain which can occur within minutes of intranasal drug administration. In the extracellular transport-based route, the administered substances could first cross the gaps between the olfactory neurons in the olfactory epithelium which are subsequently transported into the olfactory bulb. In the case of the extra-cellular transport-based route, the administered substances may be transported along the trigeminal nerve to bypass BBB. After reaching the olfactory bulb of the trigeminal region the substances may enter into other regions, of the brain by diffusion, which may also be facilitated by perivascular pump that is driven by arterial pulsation.[4] Thus, this study is aimed at transporting the active substance via nasal route to produce action in brain.

MATERIALS AND METHODS

Materials

Atomoxetine HCl was obtained as gift sample from Maan Medix Pharma Ltd, poloxamer-407 and HPMC K100 were procured from Yarrow Chemicals Ltd, carbopol-934P from S.D. Fine Chemicals Ltd. All other reagents and chemicals used were of analytical grade.

Methods

Preparation of mucoadhesive thermos-reversible nasal gels. *In situ* gels were prepared by cold method reported by Schmolka. In this method, weighed amount of thermo-reversible polymer was slowly added to cold water (4 ± 2°C) in a 250 ml beaker containing a magnetic stirring bar, stirring at a speed of 500 rpm for 2 hrs. The temperature of water was maintained at 4 ± 2°C throughout the preparation. This solution was kept overnight in the quiescent state in the refrigerator at 4°C to affect the complete dissolution of the polymer. Then, mucoadhesive agent, preservatives were added to the dispersion with continuous stirring, and then weighed amount of the drug was mixed in the above dispersion. The final volume was made up 100% with distilled water.[5]

Preliminary studies. Preliminary studies were carried out in order to find out the drug-excipient compatibility and also to optimize the concentration of thermo-reversible gelling agent.

Optimization using Taguchi OA L9 experimental design. Taguchi OA experimental design was employed for studying the effect of independent variables on response (gelation temperature). Based on preliminary studies three factors were determined: concentration of thermos-reversible gelling agent (Poloxamer 407 (19%, 20%, 21%)) type and concentration of mucoadhesive agent (C-934P, HPMC K100 and HPMC K4M at three levels of 0.2, 0.3 and 0.4%) (Table 1). An L9 orthogonal array was employed for choosing the best and optimized formulation. Taguchi experimental trials were shown in tables 2 and 3. The software used was Minitab-17 English. The resultant

formulations were studied for various evaluation parameters.[6]

Physical appearance. *In situ* gel solutions were prepared and evaluated visually for clarity under black and white background.

Determination of pH. pH of all formulations was determined by using digital pH meter, which was calibrated using buffers of pH 4 and 7 before the measurements. pH of each formulation was measured in triplicate and average values were calculated.

Table 1. Taguchi L9 orthogonal array (3^3) design of experiment.

Independent variables	Level 1	Level 2	Level 3
Factor A	19%	20%	21%
Factor B	C-934P	HPMC K4M	HPMC K100
Factor C	0.2%	0.3%	0.4%

Table 2. Taguchi L^9 orthogonal array (3^3) permutation combinations.

	Independent variables		
Trials	Factor A	Factor B	Factor C
1	1	1	1
2	1	2	2
3	1	3	3
4	2	1	2
5	2	2	3
6	2	3	1
7	3	1	3
8	3	2	1
9	3	3	2

Where Factor A- Concentration of gelling agent; Factor B- Type of mucoadhesive agent; Factor C- Concentration of mucoadhesive agent

Table 3. Taguchi experimental runs formulae.

Material (w/v)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Atomoxetine HCl (mg) (10 mg/ml)	250	250	250	250	250	250	250	250	250
Poloxamer-407 (g)	4.75	4.75	4.75	5	5	5	5.25	5.25	5.25
Carbopol 934P (mg)	50	-	-	75	-	-	100	-	-
HPMC K4M (mg)	-	75	-	-	100	-	-	50	-
HPMC K100 (mg)	-	-	100	-	-	50	-	-	75
Distilled water	Up to 25 ml								

Determination of sol-gel temperature (sol-gel). Gelation temperature was determined by Miller Donovan technique. 2 ml of the solution was transferred to test tube and was immersed in water bath of low temperature (4-5°C) and was heated at a rate of 1°C/min and left to equilibrate for 5 mins at each new setting. Gelation was deemed to occur when the meniscus would no longer move upon tiltingto 90°C. [7]

Determination of gelling capacity. Gelling capacity was determined based on the formulation behaviors like gelling time and erosion time of formed gel due to the environmental changes[8]

+- 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.

Drug content. Formulation equivalent to 10 mg of drug was diluted with phosphate buffer of pH 6.4. After suitable dilutions, the drug concentration was determined at 273 nm using UV visible spectrophotometer and the drug concentration was calculated using the formula.[9]

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

Viscosity of formulation at solution state and gel state. Viscosity was measured at 25°C and 37°C using Brookfield viscometer with spindle number 62 at 50 rpm. Initially, viscosity of gel solution was measured and then this solution was allowed to convert to gel by increasing the temperature of the solution with the help of water bath whose temperature was maintained at 37±2°C. Formulation containing Carbopol, pH was also increased along with temperature. Then the viscosity of this formed gel was measured.[10]

Gels strength. An accurately weighed quantity (10 g) of gel was placed in a 25 ml graduated measuring cylinder and was allowed to form a gel. A weight of 5 g was placed on to the gel, the time taken by the weight to sink 5 cm down through the gel was measured.[11]

Mucoadhesion studies. The mucoadhesive strength of each formulation was determined by measuring the force required to detach the formulation from sheep nasal mucosal tissue using modified physical balance. The biological membrane was fixed under one pan of the balance with the help of cyanoacrylate glue using the slide and was hydrated with 100 ml of phosphate buffer pH 6.4. Accurately weighed equivalent amount of 10 mg of gel was stuck to the inverted beaker (250 ml) or another slide and the height of the balance was adjusted. A preload of 2 g was applied in order to allow the formation of mucoadhesive joints. After a 3 min rest period, the preload was removed and gradually the weight was added to the other pan until the gel was detached from the mucosal surface. The total weight required for the complete detachment of the gel was recorded.[11, 12]

$$\text{Mucoadhesive strength (dynes/cm}^2) = \frac{mg}{A}$$

Where m is weight required for detachment in gram, g is acceleration due to gravity (980 cm/s^2), and A is area of mucosa exposed

***In vitro* drug release studies.** (1) *In vitro* diffusion study using dialysis membrane: Diffusion studies were performed using Franz diffusion cell which was locally fabricated. The dialysis membrane was placed between donor and receptor compartment. Gel formulation was uniformly applied on the membrane and clamped together. The receptor compartment was filled with phosphate buffer saline , pH 6.4 and maintained by continuous stirring at 50 rpm with a magnetic bead and maintained at 37°C. At predetermined time intervals, one ml sample was withdrawn and replaced with an equal volume of buffer. The samples were analyzed after appropriate dilution at λ_{max} of 273 using spectrophotometer.[13] The release rate was calculated by plotting the amount of drug permeated versus square time.

(2) *In vitro diffusion study using egg membrane:* Following the above mentioned methodology egg membrane was used instead of the dialysis membrane.

In order to establish an *in vitro* method for determining the drug release from gels, drug permeability was investigated using egg shell membrane that has pores and channels with hydrophilic properties which permeates small to middle size drugs to diffuse in a manner similar to human skin/mucosal membranes.

(3) *Ex vivo* permeation studies using sheep nasal mucosa: *Ex vivo* drug permeation study was carried

out for best formulations. Fresh nasal tissue was removed from the nasal cavity of a sheep obtained from a local slaughter house. The mucosa was stored in saline water at frozen condition. It was placed in between the donor and the receptor compartment, and the same procedure was followed.[14]

Curve fitting analysis. Mechanism of drug release from *in situ* gel was studied by fitting the dissolution 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 drug release was determined.

RESULTS AND DISCUSSION

FTIR study. IR spectra of pure drug Atomoxetine HCl showed characteristic absorbance peak at 3442, 2954, 3608, 1242, 1010, 771 indicating Ar-H stretching, C-H stretching, C-N stretching, Ar-O-R stretching, R-NH stretching, C-O stretching. The peaks identified in the pure drug were relatively same when compared with the polymer blend, indicating no drug polymer interaction *i.e.* the pure drug was not altered functionally and is compatible with polymers.

Optimization of concentration of poloxamer. Different concentrations of poloxamer were screened to get thermo-reversible gel on the basis of gelation temperature. There was an inverse correlation seen between poloxamer concentration and time required for the formation of gel and gelation temperature. From the screening studies, 19, 20, 21 percentages of poloxamer were selected for further studies to apply Taguchi OA experimental design. Selected percentages of poloxamer were further screened with the addition of mucoadhesive agents for gelation temperature. Combination studies revealed that the addition of the mucoadhesive agents also affected the gelation temperature. As the concentration of the mucoadhesive agent was increased the gelation temperature decreased and time required for gelling also decreased.[15]

Physical appearance. Gel base was liquid at 4°C and formed gels at the body temperatures. Formulations with poloxamer 19% were semi-stiff, 20% and 21% were stiff, which indicates an increase in the concentration of poloxamer, stiffer is the gel formed. Formulations containing carbopol were white, opaque while HPMC formulations were white and transparent.

pH. Physiological pH of nasal mucosa is 4.5-6.5. However, nasal mucosa can tolerate within the pH range of 3-10. pH for all the formulations was in the range of 4.9 to 6.9 (Table 4) indicating non-irritancy of formulations. 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.[5]

Gelation temperature. Gelation temperature range suitable for nasal gel was 32-37°C. Gelation temperature for all the formulations was found to be in the range of 33-39°C (Table 4). Increase in the concentration of poloxamer decreased gelation temperature. The decreased gelation temperature was owing to the interaction between the hydrophobic portion of polymer molecule, which could disrupt micelle structure and increase 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. Formulations F4 to F6 exhibited transition temperatures within the range appropriate for drug delivery.[16]

Gelling capacity. Formulations F1 to F3 gelled after few minutes and dissolved rapidly, while F4 to F5 converted to gel after few minutes and remained intact for few hours, on the other hand, formulations F6 to F9 showed immediate gelation and remained intact for an extended period of time (Table 4).

Gelling strength. Gelling strength increased with the increase in the concentration of the gelling agent, formulations F1, F2, F3 showed lesser strength than F4, F5, F6 followed by F6, F7, F8 formulations, owing to the micellar nature of poloxamers which is made up of micellar subunits of cubic orientation and the formation of tight interactions due to excessive

hydrogen bonding between water molecule and ethereal oxygen of the polymer a phenomenon related to tightly packed cubic liquid crystalline micellar phases. As the concentration of polymer increases, the gel structure becomes closely packed with the arrangement in the lattice pattern (Table 4). The mucoadhesive agent added also showed the effect on gel strength greater the percentage of mucoadhesive agent, greater was the gel strength seen.[17]

Table 4. Results of evaluation parameters for the taguchi experimental batches (*n*=3).

FC	pH ± SD	Gelation temp. ± SD (degree)	Gelation time ± SD (mins)	Gelling capacity	Gelling strength (mins) ± SD	Viscosity of sol-state ± SD(CPS)	Drug content ± SD	Mucoadhesive strength ± SD (dynes/cm^2)
F1	4.9 ± 0.03	39°C ± 0.2	3.52 ± 0.06	+	2.2±0.03	89.13±0.13	97.31 ± 0.25	1721 ± 0.12
F2	6.8 ± 0.19	39°C ± 0.3	3.41 ± 0.02	+	2.4±0.05	111.61±0.65	96.07 ± 0.27	1321 ± 0.24
F3	6.9 ± 0.02	39°C ± 0.5	3.2 ± 0.04	+	2.9±0.06	92.93 ±0.75	97.3 ± 0.94	1541 ± 0.32
F4	4.8 ± 0.04	37°C ± 0.4	2.11 ± 0.02	++	3.46±0.05	121.91±0.03	98.34 ± 0.07	1998 ± 0.41
F5	6.9 ± 0.05	36°C ± 0.3	1.26 ± 0.04	++	3.57±0.09	158.56±0.05	96.47 ± 0.06	1724 ± 0.53
F6	6.7 ± 0.03	37°C ± 0.2	2.4 ± 0.01	++	3.51±0.05	146.27±0.32	98.33 ± 0.04	1623 ± 0.21
F7	4.5 ± 0.08	34°C ± 0.2	2.34 ± 0.03	+++	4.45±0.02	136.91±0.24	97.33 ± 0.52	2419 ± 0.52
F8	6.6 ± 0.06	34°C ± 0.6	1.31 ± 0.07	+++	4.32±0.01	189.52±0.54	96.8 ± 0.43	2119 ± 0.62
F9	6.4 ± 0.04	33°C ± 0.7	1.23 ± 0.02	+++	4.28±0.04	178.41±0.21	96 ± 0.33	1976 ± 0.31

FC: Formulation code

Viscosity of sol-state. Viscosity increased with the increase in the concentration of the gelling agent. Formulations F1, F2, F3 showed lesser viscosity than F4, F5, F6, followed by F6, F7, F8, and there was a decrease in viscosity with an increase in temperature, but variation seen due to combination of polymers (Table 4). This indicated the formation of temperature induced gel structure of poloxamer. All the formulations remained liquid up to certain temperature and withan 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 changes in viscosities were observed due to a sudden rise in micellar concentration at higher temperatures.[18]In addition to this, the mucoadhesive agent showed similar viscosity enhancing effect with an increase in its concentration. Among all, the formulations with HPMC K4M showed higher viscosity.

Drug content. Content of the drug ranged from 96 to 98.34%. F4 and F6 formulations were found to have maximum drug content which indicated the efficient loading and uniform distribution of drug throughout the gel (Table 4).

Mucoadhesive strength. Poloxamer-407 and mucoadhesive agent had shown effect on mucoadhesive strength. 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 (Table 4). 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 formation of a strengthened 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 the 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.[5, 19]

***In vitro* drug release using dialysis membrane.** *In vitro* drug release profiles were showed in Figure 1. Formulations F1, F2 and F3 exhibited 96.38, 92.58 and 95.38% of drug release in 3 hrs, respectively. Formulations F4, F5 and F6 exhibited 99.38, 94.38 and 98.58% of drug release in 4 hrs, respectively. Formulations F7, F8 and F9 exhibited 89.38, 84.78 and 87.58% of drug release in 4 hrs, respectively (Table 4). From the above results, it was concluded that as the concentration of polymers increased, there was a decrease in the drug release rate seen. 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, dimensionsand increase in the size of micelles in the gel structure.[20,21] 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.[22] Apart, these polymers were also known to express a high Ca^{2+} binding ability, and also in an increase interaccessibility 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. Hence the Formulation F4, F5, F6 containing 20% poloxamer, with 0.3% carbopol 934P, 0.4% HPMC K4M, 0.2% HPMC K100, respectively were able to release the drug for 4 hrs and selected for further studies.

***In vitro* diffusion studies using egg membrane.** The *in vitro* drug release profiles were showed in Figure 2. The formulations F4, F5 and F6 were selected based on the drug release from the dialysis membrane, these exhibited 92.1, 90.1 and 95.2% of drug release in 4 hrs, respectively (Table 4). There was a decreased drug release from egg membrane when compared to the diffusion across dialysis membrane which may be due to decrease in the diameter of pore size of the egg membrane.

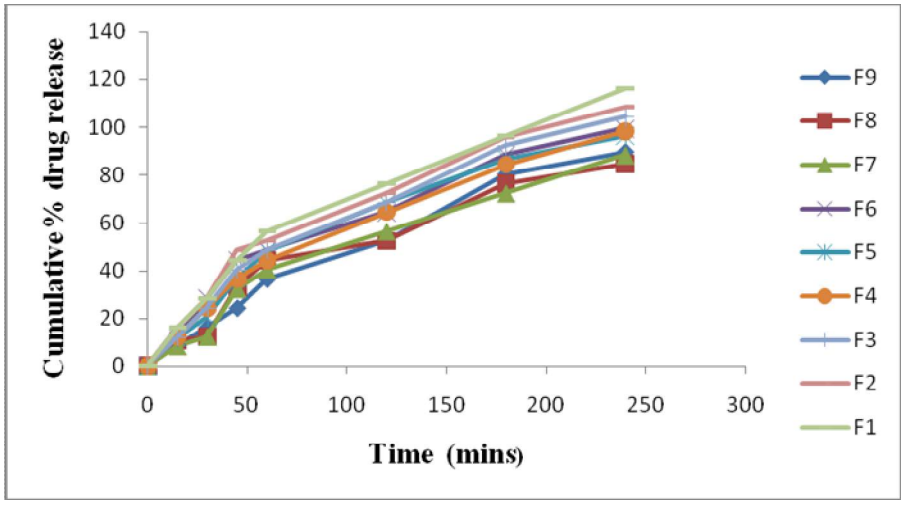

Figure 1. Cumulative percentage drug release of taguchi experimental batches

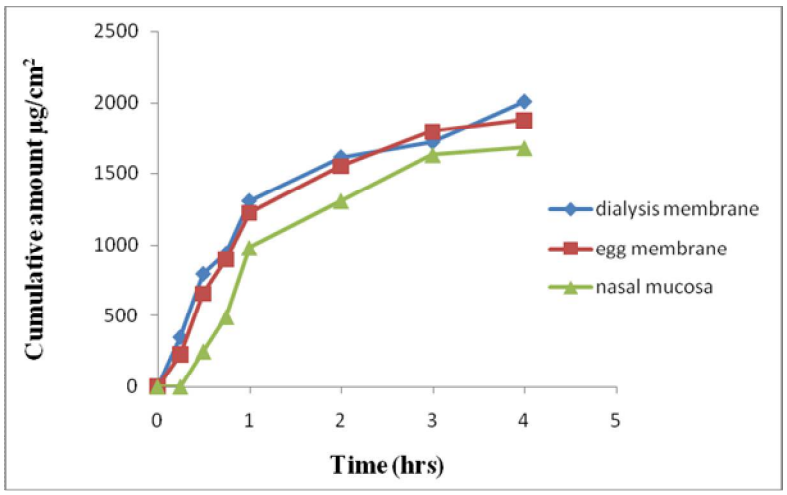

Figure 2. Comparative drug diffusion profiles of formulation F4 through various membranes

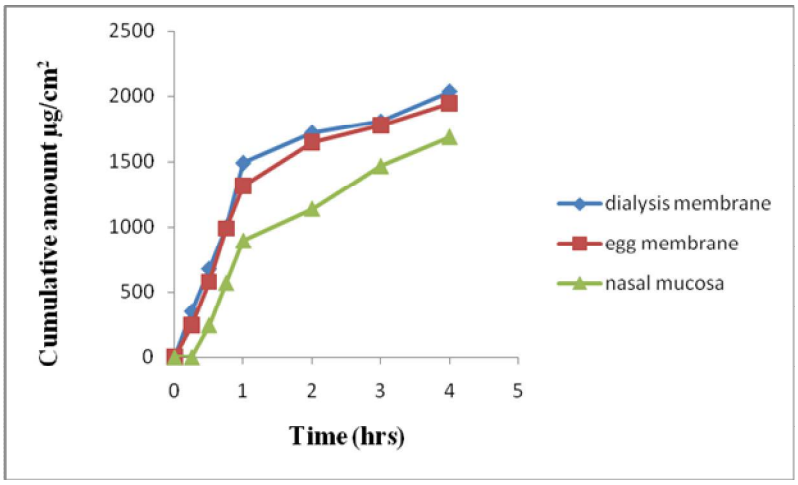

Figure 3. Comparative drug diffusion profiles of formulation F6 through various membranes

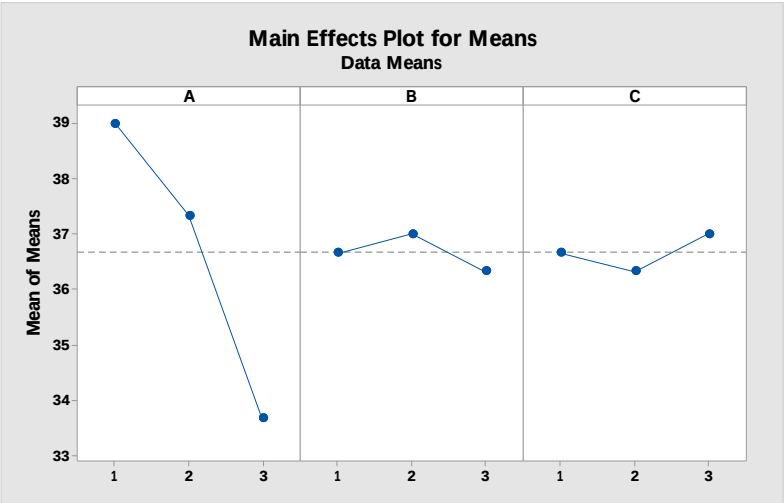

Figure 4. Main effect plot for Means

Table 5. Permeability parameters and model dependent kinetic analysis of optimized formulations (n = 3).

F C	Flux (μg/cm²/hr)	Permeability coefficient (cm/hr)	r²				N	Drug transport mechanism
			Zero	First	Higuchi	Peppas		
F4	436.9 ± 0.32	43.6 ± 0.32	0.915	0.923	0.942	0.878	0.812	Anomalous
F6	428.1 ± 0.99	42.8 ± 0.99	0.884	0.890	0.992	0.871	0.865	Anomalous

Table 6. ANOVA table for gelation temperature vs factors A,B,C.

Source	Degrees of freedom	Sum of squares	Mean of squares	P
Factor A	2	44.667	22.3333	0.01
Error	6	1.333	0.2222	
Total	8	46.000		
Factor B	2	0.6667	0.3333	0.957
Error	6	45.3333	7.5556	
Total	8	46.000		
Factor C	2	0.6667	0.3333	0.957
Error	6	45.3333	7.5556	
Total	8	46.000		

***Ex vivo* permeation studies.** These studies were performed for optimized formulations (F4 and F6) from Taguchi experimental batches, using sheep nasal mucosa to know the amount of drug that has actually diffused across the tissue over the period of time. Ex-vivo drug release profiles for the optimized formulations were showed in figure 3. It was observed that formulations F4 and F6 exhibited drug release of 90.4 and 89.18 with a flux of 436.9 ± 0.32 and 428.1 ± 0.99 μg/cm^2/hr, respectively (Table 4), drug release from nasal mucosa when compared to dialysis membrane and egg membrane, was less and the reason was attributed to high thickness of nasal mucosa.

Drug release kinetics. Results obtained from ex-vivo release studies of the optimized batches (F4 and F6) was attempted to fit into various mathematical models. Regression coefficient r^2 values of zero order, first order, Higuchi matrixes, Peppasare tabulated in the (Table 5). Drug release from the optimized formulations was found to follow first order release kinetics with Higuchi mechanism. *In vitro* dissolution data when fitted to Korsemeyer Peppas equation, drug release was by anomalous transport. Anomalous diffusion or non-Fickian diffusion refers to combination of both diffusion and erosion-controlled rate release. Release process involves the penetration of water into the gel followed by swelling of the polymer and diffusion of the drug dissolved in the matrix.[23]

Analysis of the results using Taguchi design. The obtained results (gelation temperature data) *i,e.*, the response was analysed by Minitab 17 English software. Signal to noise ratio (S/N) for 'nominal is the best' characteristic was chosen since the goal is to take the nominal response.[23]From main effect plot of means, it can be inferred that factor A (concentration of Poloxamer) has the greatest influence on gelation temperature displayed in Figure 4. The other 2 factors B and C (a type of mucoadhesive agent, the concentration of mucoadhesive agent) had equal influence on the response. Therefore, the order of independent variables on which gelation temperature depends was, concentration of gelling agent> concentration of mucoadhesive agent and type of mucoadhesive agent. One-way ANOVA was used in the analysis of Taguchi design of experiment (Table 6). ANOVA was used to determine whether factors were significantly related to the response. It was found that concentration of the gelling agent (Poloxamer-407) has a significant effect on the

gelation temperature as the 'p' value is 0.01 at 95% confidence interval ($p < 0.05$).

CONCLUSION

Thermo-reversible nasal *in situ* gel of atomoxetine HCl was formulated using Poloxamer-407 as a thermos-gelling polymer and muco-adhesion to increase nasal residence time, was achieved by using optimized concentration of carbopol 934P, HPMC K100. Mucoadhesive strength was found to be increased with increase in the concentration of mucoadhesive polymers. Drug release from the in-situ gel was found to be increased in the order of dialysis membrane > egg membrane> nasal mucosa.

Atomoxetin HCl being a hydrophilic drug may follow the paracellular route. The underlying mechanism could be drug reaching olfactory bulb by bypassing the blood-brain barrier. Various studies have confirmed that nasal formulation follows extra-cellular transport mediated routes and may pave rapid entrance of drug into the brain which can occur within minutes of intranasal drug administration. It may diffuse into the brain and same could be predicted for this drug too. Thus, developed atomoxetine HCl nasal formulation could be used effectively to increase residence time by mucoadhesion in nasal tract thereby eliminating first-pass effect improving the bioavailability of formulation for the treatment of ADHD. Further In vivo studies on animals or human need to confirm the same.

CONFLICT OF INTEREST

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REFERENCES

1. Kailas mali, K., ShashikantDdhawale, C. and Remethdias, J. 2015. Nasal mucoadhesive in-situ gel of granisetron hydrochloride using natural polymers, *J. Appl. Pharm. Sci.* **7**, 84-93
2. Paul,A., K.M. and Nair S.C. 2017. Intra nasalinsitu gelling system of lamotrigene using ion activated mucoadhesive polymer. *Open Med. Chem. J.* **11**, 222-244.
3. Indravadan A., Shedge S., Manohar V., Wadgaonkar P. and Ashish K. 2017. Thermo-reversible sol-gel transition of aqueous solutions of patchy polymers. *Royal Soc. Chem.* **7**, 5101-5110
4. Diana Gomez, A., Jose Martinez, R., Leah Hanson, H., William Frey, and Cory toth C. 2012. Intranasal treatment of neurodegenerative diseases and stroke, *Frontiers in Biosci.* S**4**, 74-89.
5. Rita Maithiya, J., Pradip Ghosh, K., Manish Umrethia, L. and Rayasa Murthy, S.R. 2006. Thermoreversible mucoadhesive gel for nasal delivery of Sumatriptan. *AAPS Pharm. Sci. Tech*. **7**, E1-E7
6. Swathi, G. and Lakshmi, P.K. 2015. Design and optimization of hydrodynamically balanced oral in-situgel of glipizide, *J. Pharm. Pharm. Sci.* **5**, 31-38.
7. Panchal D., Patel U. and Bhimani B. 2012. Nasal in-situ gel: a novel drug delivery system. *Int. J. Pharma. Res. Scholars*. **1**, 457-473.
8. Athare, A.V., Rohamare, P., Bansode, A., Mahale N. and Chaudhari, S. 2012. Formulation and evaluation of eletriptan hydrobromide thermoreversible nasal *in situ* gel. *Int. J. Pharma. Res. Dev.* **4**, 267-275.
9. Binu Chaudhary and Suraj pal Verma. 2014. Preparation and evaluation of novel in-situ gels containing acyclovir for the treatment of oral herpes simplex virus infections. *Hindawi Publishing Corpor. Sci. World J.* 1-7. Article ID 280928.
10. Alpana, P.K., Sarfaraz, K.A.K. and Mohammed, H.D. 2012. Evaluation of Polaxomer based in-situ gelling system of articaine as a drug delivery system for anesthetizing periodontal pockets an *in vitro* study. *Indian J. Dentistry*. **3**, 201-208.
11. El-Houssieny, B.M. and Hamouda, H.M. 2010. Formulation and evaluation of clotrimazole from Pluronic F127 gels. *Drug Discov. Therapeut.* **4**, 33-43.
12. Nisha G.S., Maithili P. and Charyulu R.N. 2012. Formulation and development of nasal in-situ gels of triptans for antimigraine activity. *Int. J. Res. in* Pharm. and Biomed. Sci. **3**, 861-868.
13. Swamy, N.G.N. and Zaheer Abbas. 2012. Mucoadhesive in situ gels as nasal drug delivery systems: an overview. *Asian J. Pharma. Sci.* **7**, 168-180.
14. Aditya, G., Gannesh, K.G. and Manasa, B. 2012. Design and evaluation of controlled release mucoadhesive buccal tablets of lisinopril. *Int. J. Curr. Pharm. Res*. **2**, 24-27.

15. Hyun, J.C., Prabhakar Balakrishnan and Chang-koo Shim. 2011. Poloxamer/Cyclodextrin/Chitosan-based thermo-reversible gel for intranasal delivery of fexofenadine hydrochloride. *Indian J. Pharm. Sci.* **100,** 681.
16. Sangram Sathe, Bagade, M.Y., Nandgude, T.D., Kore K.J. and Shete R.V. 2015. Formulation and evaluation of thermo reversible in situ nasal gel of terbutalinesulphate. *Indo American J. Pharm. Res*. **31**, 3680-3687.
17. Sandeep, S. and Warade Swaroop, L. 2010. In-situ gel formulation of Ornidazole for treatment of periodontal disease. *Curr. Pharm. Res*. **1**, 60-70.
18. Parmar, V. and Lumbhani, A.N. 2012. Development and evaluation of ion-dependent in-situ nasal gelling systems of metoclopramide hydrochloride as an antimigraine model drug. *Int. J. Latest Res. Sci. Technol.* **1,** 80-89.
19. Ashok Kumar Rajpoot, Shubhini Saraf, A., Manisha, P., and Koshy, M.K. 2011. Mucoadhesive, thermosensitive prolonged release in situ nasal gel of domperidone. *Int. J. Drug Formul. Res.* **2**, 210-224.
20. Miller, S.C and Drabik, B.R.1984. Rheological properties of Poloxamer vehicles. *Int. J. Pharm.* **18**, 269-276.
21. Gowda, D.V., Tanuja, D. and Mohammed Khan, S. 2011. Formulation and evaluation of in-situ gel of diltiazem hydrochloride for nasal delivery. *Scholars Res. Library der Pharmacia Lettre*. **3**, 371-381.
22. Bignotti, F., Penco M., Sartore, L., Peroni I., Mendichi, R., Casolaro, M. and Damore A. 2010. Synthesis, characterization and solution behavior of thermo and pH-responsive polymers bearing L-leucine residues in the side chains. *Polymer*. **41**, 8247-8256.
23. Indira, T.K., Lakshmi, P.K. and Balasubramaniam, J. 2012. Enhancement of bioavailability of fenofibrate using alpha-tocopherol and phospholipids: an approach to optimize the formulations Taguchi OA L15 array design. *J. Pharmacy Pharm. Sci.* **12**, 1-8.