Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):324-331

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In situ gel based on gellan gum as new carrier for nasal to brain delivery of venlafaxine hydrochloride: *In vitro* evaluation and *in vivo* study

Inayat B. Pathan[*a], Sankat R. Nirkhe[a] and Anand Bairagi[b]

[a]*Department of Pharmaceutics, Government College of Pharmacy, Aurangabad, Maharashtra, India*
[b]*Department of Pharmacology, M. E. S. College of Pharmacy, Sonai, Ahmednagar, Maharashtra, India*

ABSTRACT

Venlafaxine hydrochloride in situ nasal gel to brain delivery to provide sustained release by improving the residence time of the drug in the nose providing rapid antidepressant effect should improve patient compliance and treatment. The aim of the present study was to develop a venlafaxine hydrochloride (VLF) nasal in situ gel based upon the concept of ion activated gelation of gellan gum for the treatment of depression. The in situ gel was characterized for drug content, gelation, viscosity, gel strength, mucodhesive strength and pH. Formulation F4 showed the effective gelation, viscosity, gel strength along with mucoadhesive strength and non-fickian diffusion process across sheep mucosa. F4 batch released 48.32 ± 2.6 % drug in 4 hours with the flux of 20.15 µg/cm^2/hr. Histopathological examinations showed no evidence of mucosal damage. The in situ gel was stable after 3 months stability study. In the pharmacodynamic FST study, the in situ gel treated rats showed significant responses (p<0.001). VLF in situ nasal gel could be potentially safe, effective and improve the residence time of the drug in the nose.

Key words: Nasal drug delivery; *In situ* gel; Gellan gum; Forced swim Test (FST); locomotor activity test (LAT).

INTRODUCTION

All phases of human life are highly affected by depression, which is mainly observed in the age group of 15-44 years in both genders [1]. Non-efficacy of antidepressant drugs are mainly due to lack of occurrence of continuous drug at the target site i.e. CNS; so sustained release antidepressant therapy is required to maintain a steady state level of drug in brain [2]. Advantage of nose to brain delivery has been reported by several inventors, such as dodging of BBB, prevention of first pass metabolism and convenience of administration and non intrusive nature [3].

Venlafaxine hydrochloride is effective in the resistant depression and is superior to selective serotonin reuptake inhibitors in preventing their occurrence of depression [4]. It has up to 30-fold greater affinity for serotonin than noradrenaline transporters [5]. It is used not only to treat depressive disorders, but also anxiety and impulsive states [6]. Venlafaxine hydrochloride is commonly available in tablet and capsule. But oral administration has a several disadvantages as it undergoes extensive hepatic first pass effect [7-8]. Hence, to control concentration within a therapeutic window and antidepressant activity in the brain, a suitable dosage form is needed.

In recent years, the nasal mucosa has exposed as an administrative route for brain targeting as well as systemic drug delivery, a desirable alternative to the parenteral medication since it is compliant to self-medication and almost painless. Rich vasculature and a highly permeable structure within the nasal membranes lead to rapid drug absorption, which offer faster onset of action as compared to oral administration [9].

In pharmaceutical and food industry natural polysaccharides and its derivatives are widely used as binding, thickening, emulsifying, and gelling agent [10]. Gellan gum is an anionic deacetylated, exocellular polysaccharide

secreted by *Pseudomonas elodea* with a tetra saccharide repeating unit of 1-β-L-rhamnose, 1- β -D-glucuronic acid and 2- β-D-glucose. The mechanism of gelation involves the formation of double-helical junction zones followed by aggregation of the double-helical segments to form a 3-D network by complexation with cations and hydrogen bonding with water [11-13]. In 1992, the USFDA approved gellan gum to be used as a food additive [14].

Gellan gum can form a transparent gel in the presence of multivalent cations, which is resistant to heat and acid [15-16].

With the recent development of novel drug delivery systems ,it is Observed that among the various exopolysaccharides, one of the most deserving polymer is gellan gum [17]. Cellulosic polymers such as hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose and sodium carboxymethylcellulose are some of the well-known mucoadhesive polymers which have been used in the development of oral, nasal, anal and vaginal dosage forms [18-20]. These polymers are biodegradable and have a high degree of mucoadhesive properties with swelling in aqueous solvents [21]. The aim of the present study was to develop a venlafaxine hydrochloride *in situ* nasal gel to provide sustained release by improving the residence time of the drug in the nose and to assess the salvation.

EXPERIMENTAL SECTION

2.1. Materials

Gellan Gum (kelcogel CG-LA) was purchased from Signet Chemical Corporation Pvt Ltd, Mumbai. Hydroxy propyl methyl cellulose (MethocelE4M) was a gift sample from Colorcon Asia Pvt Ltd., Mumbai, India. Mannitol, citric acid and propyl paraben were purchased from Merck Pvt. Ltd., Mumbai, India. Venlafaxine hydrochloride was obtained as a gift sample from Lupin Ltd., Vadodara, India. All other reagents were of analytical grade.

2.2. Method of preparation of *in situ* gels

Gellan gum was weighed and dispersed in deionised water. The dispersion was then stirred by mechanical stirrer for 30 min at 90°C on a water bath and then cooled to room temperature. Separately venlafaxine hydrochloride (7.5% w/v) and HPMC was dissolved in deionised water. HPMC solution and drug solution were added in gellan gum solution slowly with continuous stirring. An appropriate quantity of mannitol and preservative was added simultaneously. The final pH of the formulation was adjusted between 4.5 and 5.5 by using citric acid [22]. The composition of prepared formulation shown in Table 1.

Table 1. Composition of in situ gel

Ingredients	Formulation Composition (% w/v)					
	F1	F2	F3	F4	F5	F6
Venlafaxine HCL	7.5	7.5	7.5	7.5	7.5	7.5
Gellan gum	0.2	0.2	0.4	0.4	0.6	0.6
HPMC E4M	0.5	0.75	0.5	0.75	0.5	0.75
Mannitol	4.2	4.2	4.2	4.2	4.2	4.2
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02
Deionized water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

2.3 Characterization

2.3.1. Gelation studies

Gelation is the process which is evaluated by transition of liquid phase to gel [23]. 2 ml of each formulation was added in transparent glass vials containing a magnetic bar on a magnetic stirrer. The simulated nasal fluid (aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml $CaCl_2$) which had the cationic composition of nasal secretions; was added slowly while stirring [24]. Gelation point was determined when the magnetic bar stopped moving due to gelation. The consistency of formed gel was checked and graded. The experiments were repeated for thrice.

2.3.2. Viscosity and rheological behavior studies

Viscosity of formulations before and after gelation were measured by Brookfield R/S CPS + Rheometer with software Rheo 3000 and using spindle CP-75 at 100 rpm shear rate.

2.3.3. Gel strength determination

The test was performed using gel strength apparatus. Formulations (50g) were placed in a 100 ml measuring cylinder and gelation was induced by simulated nasal fluid. The apparatus for measuring the gel strength was then placed on the gel. The gel strength was measured as the time required for moving the apparatus 5 cm down through the gel [25]. Experiments were performed in triplicate.

2.3.4. pH determination
PH of the each formulation was determined by using a pH meter (Model No. CL 54, Lab India Pvt. Ltd., India). Experiments were performed in triplicate.

2.3.5. Mucoadhesive strength study
Mucoadhesive force was determined by using sheep nasal mucosa. Sections of tissue were made carefully. On glass vials, tissues were fixed in a manner that the mucosal side became outer part and properly fixed. A vial with a section of tissue was connected to the modified balance and suitable height was maintained. The gel was applied to the exposed tissue of lower vial. The height of the vial was adjusted so that the gel could adhere to the mucosal tissues of upper vial. After applying constant weight for several minutes, suitable weights were added to the modified balance [26]. Minimum amount of weight that detached two vials expressed as mucoadhesive force (dynes /cm^2).

Detachment stress (dynes /cm^2) = Mg/A
where, M is the weight added to balance in grams; g is the acceleration due to gravity taken as 980 cm/sec^2; A is the area of the tissue exposed and is equal to πr^2 (r, the radius of the circular hole in the aluminium cap).

2.3.6. Drug content
100 µl of the formulation was transferred to 50 ml volumetric flasks and final volume was made up with phosphate buffer pH 6.6 and amount of drug concentration was determined at λ_{max}225 nm using UV-visible spectrophotometer (Shimadzu, UV-1700).

2.3.7. Compatibility study
Venlafaxine-excipients compatibility study was carried out using FTIR. Spectra of pure drug and formulations (physical mixture) were obtained using a KBr pellet method (applying 6000 kg/cm^2). Each spectrum was recorded in the frequency range of 4000-450 cm^{-1} (Shimadzu FTIR Prestige-21).

2.4. *Ex vivo* permeation study
Fresh nasal tissues of sheep were obtained from the local slaughterhouse. Each side of the membrane was wiped with isopropyl alcohol to remove adhering mucous and fat and was cut to appropriate size (0.8 cm^2). Nasal mucosa was mounted on Franz diffusion cells. Phosphate buffer pH 6.6 was added to the receptor compartment. After a pre-incubation time of 20 min, pure drug solution and formulation equivalent to 7.5 mg of venlafaxine hydrochloride was placed in the donor compartment. At a predetermined time, 1-ml sample was withdrawn from the receptor compartment, replacing with fresh phosphate buffer, for a period of 240 min. The withdrawn samples were filtered, diluted and amount of permeated drug was determined using a UV-visible spectrophotometer at λ_{max} 225 nm.

2.4.1. Release mechanism
To confirm the exact drug release mechanism form gel matrices, the release data from ex vivo permeation studies was subjected to mathematical treatment using Korsemeyer-Peppa's exponential equation as follows, release mechanism operational the data were fitted according to the equation

$$M\,t / mT = k\,t^{n}$$

Where Mt/mT is the fraction of drug released, k is kinetic constant; it is release time and 'n' is the diffusional exponent for drug release. The value of 'n' gives an indication of the release mechanism; when n = 1, the release rate is independent of time (zero-order) (case II transport), n = 0.5 for Fickian diffusion and when 0.5 <n <1.0, diffusion and non-fickian transport are implicated [27].

2.4.2. Histopathological study
Histopathological examination of the optimized F4 formulation on control tissue and treated tissue of nasal mucosa was performed using a light microscope (Nikon Eclipse E600, Japan). Tissue was fixed with 10% buffered formalin (pH 7.0), routinely processed and embedded in paraffin. Sections (5 µm) were cut on glass slides and stained with hematoxylin and eosin [28].

2.5. In vivo Pharmacodynamic study
Approval to carry out pharmacodynamics studies was obtained (Institutional Animals Ethical Committee, approved the protocol). Locomotor Activity test (LAT) and Forced Swim test (FST) was used to evaluate the antidepressant effect of the optimized F4 formulation. Rats of either sex weighing 250–300 g were kept under standard laboratory conditions (temperature 23-30^{o}c). The rats were kept with free access to standard laboratory diet. Rats were

divided randomly into four groups, each containing three animals (n= 3). Group -1 was treated with saline and was considered as a control. Group-2 was treated with oral tablets of venlafaxine hydrochloride containing route containing 1.40 mg/day. Group 3 was treated with a simple venlafaxine solution containing 0.6 mg/day by the intranasal route. Group-4 was treated with optimized *in situ* gel formulation containing 2 mg/day (equivalent to 0.60 mg/day) drug. The doses were administered without anesthesia by using simple poly-ethylene tube.

2.5.1. Force swim test

Rats were forced to swim in a cylindrical glass tank (60 cm height X 30 cm in diameter) containing water after the administration of doses. The water was filled up to 40 cm height so they swam without touching their hind limb or tail to the bottom of the tank. On the 1st day of experiments, rats were forced to swim for 10 min. After 24 h, rats were re-exposed to forced swim for 5 min and animals were judged for immobility, climbing, and swimming. After a 5-min swim test, the rat was removed from the cylinder, dried and then returned to its home cage [29].

2.5.2. Locomotor activity

Hyperactivity, functional roles of specific neurobiologicals and drugs potential psychoactivity were discriminate by the locomotor activity study [30-31].

Locomotor activity was measured in the open-field test. The apparatus consisted of a square arena (200×200 cm), with a 50 cm height. The floor was divided into 30 equal squares. Animals were individually positioned in the center of the arena and the activity was measured over 5 min. The open field was cleaned with isopropyl alcohol solution before behavioral testing to avoid possible bias due to odors and/or residues left by rats tested earlier. Also, after each 3 animals apparatus was cleaned [32].

2.6. Stability study

Three batches of optimized F4 formulation were kept for stability study as per ICH guideline. A sufficient quantity of *in situ* gel stored in well closed glass container for a period of 3 months at 40 ±2°C/ 75 ±5% RH in stability chamber. The formulation was evaluated at periodical intervals of one month for clarity, drug content, viscosity and pH.

2.7. Statistical analysis of data

The results were analyzed by one-way ANOVA with turkey post *t*-test using Graph Pad Prism software-5 version (Graph Pad Software Inc., San Diego, USA).

RESULTS AND DISCUSSION

3.1. Gelation

To understand the concept of *in situ* gel formation, in vitro gel study was carried out by using artificial nasal fluid. A scale ranging between – and ++++, as shown in Table 2 was used to assess gelation. After easy instillation into the nasal cavity the liquid solutions should undergo a rapid changed from sol-to-gel transition by means of ionic gelation. Formulation (F1-F3) showed weakest gelation while (F5-F6) showed very stiff gelation.

Table 2. Physical parameters of venlafaxine HCl in situ gelling system

Formulation code	Gelation	Mucoadhesive strength	Viscosity	Gel strength	Drug content	pH
F1	+	2050±2.3	151±2	15±2	98.35±0.95	5.3
F2	+	2340±1.6	242±3	17±3	98.62±0.56	5.5
F3	++	2152±2.1	500±1.9	23±3	98.25±0.62	5.5
F4	+++	2406±1.8	540±2.2	32±3	98.32±0.40	5.6
F5	++++	2304±2.4	825±1.8	52±2	97.26±0.39	5.5
F6	++++	2608±2.1	860±3	56±3	98.27±0.87	5.5

Values are expressed in mean ±SD, n =3.
(-) No gelation,(+) weak gelation,(++) Immediate gelation remains for few hours,(+++) Immediate gelation remains for extended period (stiff gel), (++++)very stiff gel

3.2. Viscosity and rheological behavior studies

The viscosity of formulations both in sol and gel state was found to be proportionate with the increasing polymer concentration as shown in Table 2. This helps to provide longer residence time in the nasal cavity to overcome MCC.

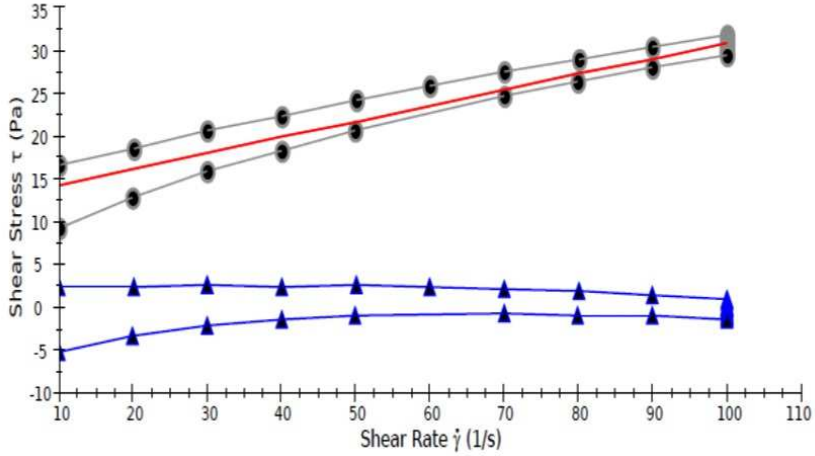

Figure.1. Shear thining behaviour of F4 formulation

3.3. Gel strength determination

Values for gel strength for all formulations were shown in Table 2. The formulations (F1- F3) showed the gel strength value less than 20 sec and formulation (F5-F6) showed the gel strength value more than 45 sec this may be due to polymer concentration. Formulation F4 showed the gel strength value in the range 35±3 sec which was acceptable.

3.4. pH determination

The nasal mucosa can tolerate solutions within a pH range of 3-10 [33]. The pH of all formulations was found to be in a range of 5.3-5.6 as shown in Table 2.

3.5. Mucoadhesive strength

The mucoadhesive strengths of all formulations are shown in Table 2. In comparison to all formulations F4 formulation showed significant (2406±1.8 dyne/cm^2) mucoadhesive strength.

3.6. Drug content

Drug content of all formulations (Table 2) was in the range 97.26±0.39 - 98.62±0.56%
(n=3, mean ± SD).

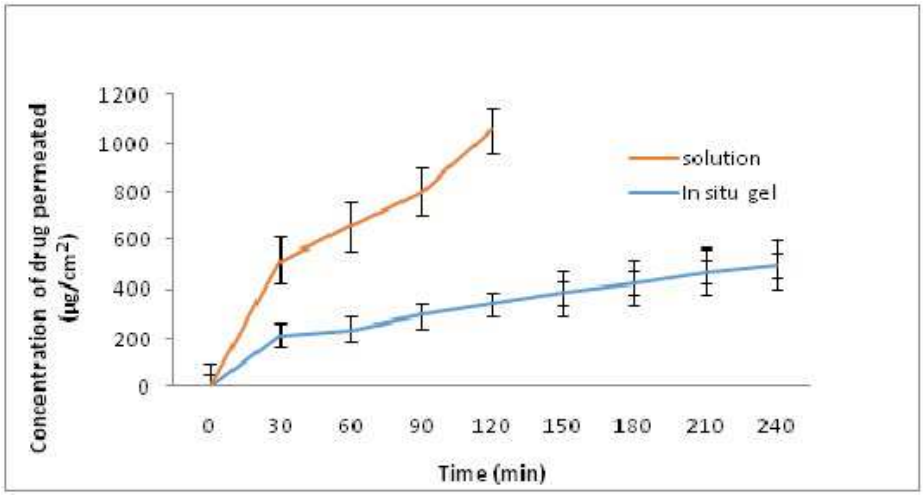

Figure.2. Percent cumulative permeation of drug across sheep nasal mucosa

3.7. Compatibility study

The FTIR of venlafaxine hydrochloride shows an intense band at 1610.56 cm^{-1}, 1442.2 cm^{-1}, 1274.60 cm^{-1} and 1037.70 cm^{-1} corresponding to the functional groups C=O, COOH, NH and OH blending. The of drug and excipients shown intense band at 1695.43 cm^{-1}, 1583.56 cm^{-1}, 1485.19 cm^{-1} and 1080.14 cm^{-1} indicates no change in the functional groups C=O, COOH, NH and OH. From the above analysis, it was found that there was no

major shifting of characteristic peaks of the drug. So, it concluded that no drug and excipients interaction were found.

3.8. *Ex vivo* permeation study

The permeability coefficient (P) was also calculated and found to be 0.019.11 cm h^{-1} while the steady state flux was 20.15±2.6 µg cm^{-2} h^{-1}. From drug release data it was investigated that drug release rate from aqueous solution was very rapid but in gel retardation of diffusion of the drug by gel matrix and prolongation of diffusion process showed that formed gel had the ability to retain drug for persistent action.

3.9. Release mechanism

The drug transport mechanism of the formulation was determined by using the Korsemeyer-Peppa's exponential equation [$(Mt/M) = K\ t\ n$]. Form the plot of log (Mt/M) vs. log of time, the kinetic parameter n and K were calculated. The observed n value was 0.67, indicating the anomalous (non-fickian) release kinetic. This indicates that the release of venlafaxine hydrochloride followed erosion- diffusion mechanism.

3.10. Histopathological study

When the nasal mucosa was treated with an optimized formulation (Fig.3A.), no significant changes were observed in the nasal mucosal morphology compared with untreated (Fig.3B.). As compared to untreated mucosa, very small degeneration with slight erosion and increased vascularity in basal membrane was observed for treated nasal epithelium. Hence, prepared *in situ* gel was safe to deliver venlafaxine through nasal route.

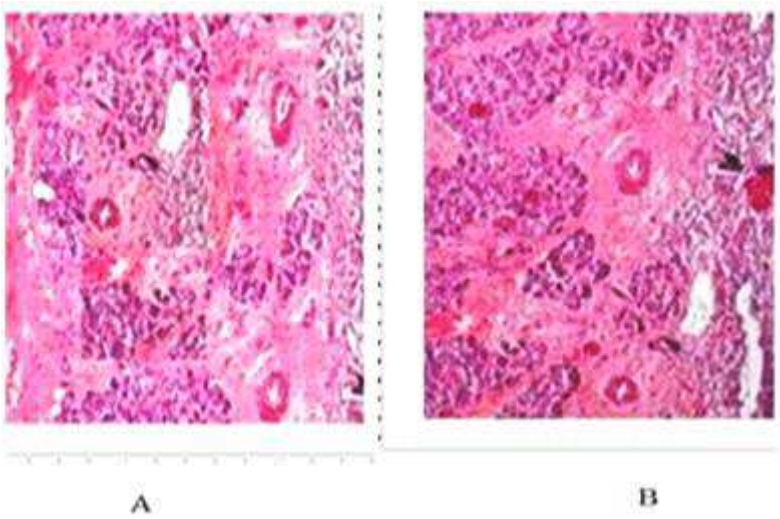

Figure. 3 Histopathological section of sheep nasal mucosa A-treated, B- untreated

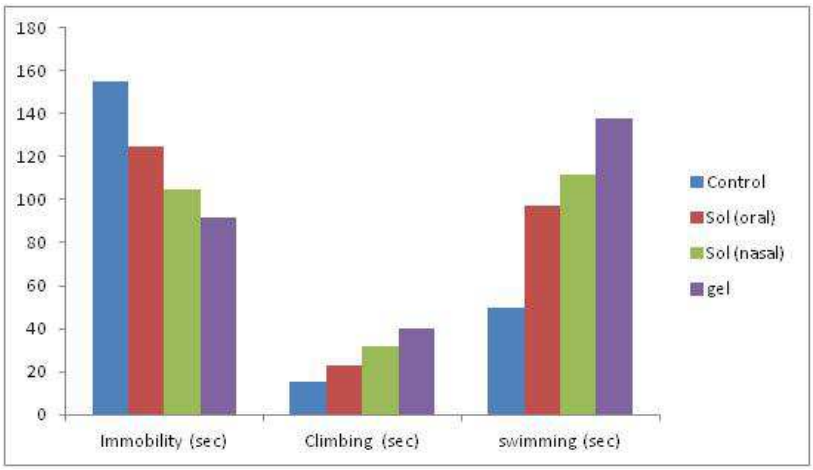

Figure. 4. Results of forced swim test

3.11. In vivo Pharmacodynamic study

3.11.1. Force swim test

Among all animal models, the FST remains one of the most used tools for screening antidepressants. Moreover, it has been reported to be reliable across laboratories [34]. *In situ* gel significantly ($p<0.001$) reduced total immobility period and significantly increase climbing and swimming behavior as compared to control, oral and nasal solutions as shown in Fig.4.

3.11.2. Locomotor activity

There was no significant ($p>0.05$) difference in the values of locomotor activity of the control, orally treated, intranasal solution and *in situ* nasal gel treated rats as shown in Table 3. The results revealed that the animals were not hyperactive. The output of both pharmacodynamic test, it can be concluded that VLF was more effective as an antidepressant by nasal route in comparison to oral administration. This may be due to the required concentration of venlafaxine hydrochloride reaches target site by the nasal route.

Table 3. Results of locomotor activity

Treatment group	No of square crossed			Mean
Control	83±2	83±4	83±2	83±2
VLF oral administration	86±3	87±3	86±3	87±2
VLF in situ nasal gel	85±2	84±3	85±2	84±3

Values are expressed in mean ±SD, n = 3 , p value < 0.05 considered statistically significant

3.12. Stability study

Based on visual identification, the *in situ* gel has remained as liquid for a period of 3 months without the occurrence of turbidity or change in color. No significant difference was observed in pH, viscosity, drug content of F4 over a period of 90 days. Therefore, it can be concluded that the developed *in situ* gel formulations were physically and chemically stable for clarity, drug content, viscosity and pH.

DISCUSSION

The inhaled hazardous particles were removed by mucociliary clearance (MCC) which involves combine action of mucus layer and cilia, so MCC rapidly removes applied dosage form from the absorption site as it imposed the main problem in nasal application. Compared to all formulations F4 showed good gelation. This indicates that as the concentration of gellan gum increases, the gelation point was increased (immediate gelation).

All formulations showed non-Newtonian (thixotrophy) flow and shear thinning behavior as shown in Fig.1. Shear thinning behavior will increase spreadability of gel [35]. Gel strength is an important factor which can prolong the post nasal drip. The *in situ* gel must have suitable gel strength so as to administer easily and can be retained at the nasal mucosa without leakage after application. The gel strength values between 25 and 50 sec were considered sufficient as reported previously [36]. The gel strength less than 25 sec may not retain its integrity and may erode rapidly while gels having strength greater than 45 sec are too stiff and may cause discomfort to the mucosal surfaces.

Residence time of any formulation in nasal cavity depends on mucoadhesive strength of the polymer. On the basis of gelation, gel strength, mucoadhesion study the F4 formulation was optimized and selected for further study. The sinus anatomy including the placement of the nasal cavity, turbinate, frontal, maxillary sinuses and histology of nasal mucosa of sheep is comparable to humans [37]. Permeation studies were carried out for F4 formulation and simple aqueous solution of venlafaxine hydrochloride as shows that Fig.2. Comparative permeation from in situ nasal gel, aqueous solution showed 95% permeation from the simple solution in 2 hours. Where as, 48.32% drug was permeated from in situ gel in the 4 hours.

CONCLUSION

The purpose of the present investigation was to develop a nasal delivery system of a venlafaxine hydrochloride based upon the concept of ion activated *in situ* gelation of gellan gum. Developed *in situ* gel formulations showed the effective gelation, viscosity, gel strength and good permeation across the sheep nasal mucosa along with mucoadhesive strength. From the histopathological study it revealed that formulation did not show any remarkable damage to the nasal mucosa. The optimized formulation also retained better stability. In vivo pharmacodynamic study demonstrated that optimized batch enhanced the antidepressant action. On the basis of these results, it was concluded that venlafaxine hydrochloride *in situ* nasal gel could be potentially safe and improve the residence time of the drug in the nose for the treatment of depression.

Acknowledgement
The authors are grateful to All India Council for Technical Education, New Delhi (AICTE) Govt. of India for providing financial assistance to I. B. Pathan under Career Award for Young Teacher.

REFERENCES

[1] CD Mathers; D Loncar. *PLoS Med*. **2006**; 3 (11), 2011–2030.
[2] CD Kilts. *J. Clin. Psychiatry*. **2003;** 64, 31–33.
[3] L. Illum. **2000**; 11, 1–18.
[4] AM Gutierrez; LG Stimmel; YJ Aiso. *Clin. Ther*. **20**03; 25, 2138–2154.
[5] GG Gould; AV Altamirano; MA Javors; AA Frazer. *Biol Psychiatry*, **2006** ; 59,408–414.
[6] MJ Millan. *Pharmacol Ther*. **2006**; 110, 135–370.
[7] SM Stahl; MM Grady; C Moret; M Briley. *CNS Spectrosc*. **2005**; 10, 732–747.
[8] L Karlsson; U Schmitt; M Josefsson; B Carlsson; J Ahlner, F Bengtsson; FC Kugelberg; C Hiemke. *Eur. Neuropsycho pharmacol***. 2010**; 20, 632–640.
[9] HR Costantino; L Illum; G Brandt; PH Johnson; SC Quay. *Int. J. Pharm*., **2007**; 337, 1–24.
[10] HS Mahajan; VK Tyagi; RR Patil; SB Dusunge. *Carbohydrate Polymers*. **2013;** 91, 618– 625
[11] H Grasdalen; Smidsroed,. *Carbohydr. Polym*. **1987;** 7, 371-393.
[12] R Chanrasekaran; LC Puigjaner; KL Joyce; S Arnott. *Carbohydrate Research*, **1988;** 181, 23–40.
[13] R Chanrasekaran; VG Thailambal. *Carbohydrate Polymer*, **1990**; 12, 431–432.
[14] DE Pszczola. *Food Technology*, 1993; 47, 94–96.
[15] GR Sanderson. In P. Harries (Ed.), Food Gels New York: Elsevier Science. **1990**; 201–232.
[16] G Sworn. Gellan gum. **2000**; 5, 117–136.
[17] AB Dawalbaje; SB Dhuppe; and SS Mitkare. *Inventi Impact: Novel Excipients*, **2012;** 40, 345–370
[18] D Schmaljohann. *Advanced Drug Delivery Reviews*, **2006**; 58(15), 1655–1670.
[19] K Bansal; MK Rawat; A Jain; A Rajput; TP Chaturvedi, S Singh. *AAPS Pharm.Sci.Tech*, **2009**; 10(3), 716–723.
[20] ID Gonjari; AH Hosmani; AB Karmarkar; AS Godage; SB Kadam; PN Dhabale. *Drug Discoveries and Therapeutics*, **2009**; 3(1), 6–9.
[21] X Jiang; S Cao; E Chen; Q Zhang. *Acta Pharmacol Sinica*. **2007**; 28(4), 584–590.
[22] J. Balasubramaniam; S Kant, JK Pandit. Acta Pharm, **2003**; 53, 251–261.
[23] PJ Watts, P.J., Illum, L. *US Patent*: **2002**; 6,432,440.
[24] CS Yong; JS Choi; QZ Quan; Rhee, J.D., Kim, C.K., SJ Lim; KM Kim; Oh, P.S., Choi, H.G. *Int J Pharm*. **2001**; 226, 195–205.
[25] NM Zaki; GA Awada; ND Mortada. *Eur J Pharm. Sci*. **2007**; 32:296–307.
[26] RW Korsemeyer; R Gurny; EM Doelker; P Buri; NA Peppas. *Int. J. Pharm*, **1983**; 25-35.
[27] RSR Murthy, RJ Majithiya; PK Ghosh, et al. *AAPS. Pharm Sci Tech*.; **2006**; 7(3):E1–7.
[28] A Dhir; SK Kulkarni. *Eur J Pharmacol*. **2007**; 568(1-3):177-85
[29] MP Paulus, SC.Dulawa, RJ.Ralph, MA.Geyer,. *Brain research*, **1999**; 835, 27-36.
[30] RD Porsolt; A Bertin; M Jalfre. *Arch Int Pharmacodyn Ther*. **1977**; 229, 327–336.
[31] N Mygind; R Dahl. *Adv Drug Del Rev*. **1998**; 29, 3–12.
[32] CP Pujara; Z Shao; MR Duncan; AK Mitra. *International Journal of Pharm*. **1995**; 114, 197-203.
[33] B Petit-Demouliere; F Chenu; M. Bourin. *Psychopharmacology* **2005;** 177(3) 245-255.
[34] DH Owen; JJ Peters; DF Katz. *Contraception***. 2006**; 2, 321–326.
[35] HS Mahajan; SG Gattani. *Drug Delivery*. **2010**; 17, 19–24.
[36] L Illum. *J. Drug Targeting*. **1996**; 3, 427–442.
[37] RA Gross; B Kalra. *Biodegradable polymers for the environment. Science*, **2002**; 297(5582), 803–807.