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FORMULATION AND EVALUATION OF *IN-SITU* MUCOADHESIVE NASAL GEL OF VENLAFAXINE HYDROCHLORIDE

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Keywords:

Nasal Gel, Venlafaxine Hydrochloride, Carbopol 940, Mucocilliary Clearance

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ABSTRACT

The prolonged residence of drug formulation in the nasal cavity is of most importance for intranasal drug delivery. The objective of the present investigation was to develop a mucoadhesive in situ gel with reduced nasal mucocilliary clearance in order to improve the bioavailability of the antidepressant drug, Venlafaxine Hydrochloride. Hence it was planned to formulate in situ nasal gel of Venlafaxine Hydrochloride for systemic delivery. Xanthan gum was used as a natural mucoadhesive polymer to formulate nasal in situ gel of Venlafaxine Hydrochloride to sustain the release of drug, to reduce mucociliary clearance thereby increasing the contact of formulation with nasal mucosa and hence improving the absorption of drug. Carbopol 940 was key ingredient which gives pH induced sol gel conversion of formulations. These formulations were evaluated for pH, drug content, viscosity, gel strength, mucoadhesive strength, *in vitro* drug release and *in vitro* permeation profile. A 3^2 full factorial design was applied to study effect of varying concentration of independent variables Carbopol 940 (X1) and Xanthan gum (X2) on dependent variables in vitro drug release, viscosity and mucoadhesive strength. In vitro drug release kinetics was studied using different kinetic models to know exact mechanism of drug release. It was found that formulation additives shows effect on drug release, viscosity and mucoadhesive strength, as the concentration of polymers increases mucoadhesive strength and viscosity increases, drug release was also increases.

INDRODUCTION

Today, nasal drug delivery is receiving much attention from the pharmaceutical industry. About 2 % of the overall drug delivery is administered via the nasal route. A survey among decision makers in the pharmaceutical industry highlights the importance of this delivery mode. A transmucosal drug delivery route (which includes the nasal route) can target tissue and use active transdermal processes. It is also regarded as a major influence of the market.In recent years many drugs have been shown to achieve better systemic bioavailability through nasal route than by oral administration. [1] Nasal therapy, has been recognized form of treatment in the Ayurvedic systems of Indian medicine, it is also called "NASAYA KARMA". [2] Nasal drug delivery which has been practiced for thousands of years has been given a new lease of life. It is a useful delivery method for drugs that are active in low doses and show no minimal oral bioavailability such as proteins and peptides. One of the reasons for the low degree of absorption of peptides and proteins via the nasal route is rapid movement away from the absorption site in the nasal cavity due to the mucociliary clearance mechanism. [3]

Venlafaxine hydrochloride, an antidepressant, is used in treatment of major depressive disorder, social anxiety disorder, generalized anxiety disorder and panic disorder. Drug is extensively metabolized in the liver via CYP2D6 and so has low oral bioavailability (45%). [4] Under these circumstances, intranasal delivery appears to be an attractive alternative. However, low residence time of drug in nasal cavity affect absorption and in turn bioavailability of drug. Hence, the design of nasal dosage forms has to consider the anatomic and physiologic characteristics of nasal mucosa and more particularly the rapid mucocilliary clearance (MCC) that limits the time available for drug absorption from the applied dosage form.[5,6] So, the possible strategy to decrease rapid MCC, is the use of mucoadhesive formulations to prolong the residence time at the nasal absorption site and thereby facilitate the uptake of the drug. Ordinary gels are difficult to administer, and an accurate drug dose cannot be measured while mucoadhesive powders are not highly favoured products. They can cause irritation to the nasal mucosa and give a gritty feel to the tissues.[7] A nasal mucoadhesive in situ gel appears very attractive since it is fluid-like prior to nasal administration and can thus easily be administered as a drop allowing accurate drug dosing. In situ gelation can be achieved by using thermo sensitive smart polymers which by sensing nasal temperature forms gel on instillation.[8] In order to formulate pH induced in situ gel for

nasal administration, pH induced polymer must have sol gel in the nasal mucosal surface pH range (4.5-6.5). Carbopol has excellent pHgelling property,low toxicity and irritation, excellent water solubility, good drug release characteristics, and compatibility with other chemicals. The pHinduced gelation of Carbopol has been explained on the basis that the polymer exists as a mobile viscous liquid at room temperatures but forms a rigid semisolid gel networkafter (contact with nasal surface) nasal administration. The objective of the present study was to develop Venlafaxine Hydrochloride pH induced mucoadhesive in situ nasal gel which would enhance nasal residence time and to improve bioavailability of the drug as compared with oral route.

Materials

Venlafaxine Hydrochloride was provided by JCPL Pharma, Jalgaon. Xanthan gum was provided by Signet Chemicals. Carbopol 940 (Loba Chemicals Pvt. Ltd.) and polyethylene glycol 400, Methylparaben of analytical grade were used.

Methods

Determination of λmax of Venlafaxine Hydrochloride [9]

The UV spectrum of Venlafaxine Hydrochloride was obtained using UV Jasco V630. Venlafaxine Hydrochloride (10mg) was accurately weighed and transferred to 100 ml volumetric flask. It was then dissolved and diluted up to 100 ml with distilled water. The above made solution was further diluted to obtain concentration of 20µg/ml. The resulting solution was scanned from 200-400 nm and the spectrum was recorded to obtain the value of maximum wavelength. The λmax was found to be 224 nm.

Drug excipients compatibility study[10]

Infra-red spectrum

The infra-red spectrum of Venlafaxine Hydrochloride was recorded with KBr disc over the wave number of 4000 to 400 cm-1 by using Fourier Transform Infra-red spectrophotometer [8400S Shimadzu. Japan]

Differential scanning calorimetric studies

Thermal analysis was performed using a differential scanning calorimetric equipped with a computerized data station. The sample of pure drug was weighed and heated at a scanning rate of 10°C/min between 40 and 300°C and 40 ml/min of nitrogen flow. The differential scanning calorimetric analysis gives an idea about the interaction of various materials at different temperatures. It also allows us to study the possible degradation of the material [Mettlar Toledo].

Preparation of in situ nasal mucoadhesive gel of Venlafaxine Hydrochloride [11]

In situ gels were prepared by cold technique, reported by Schmolka. To the 2.5%w/v, solution of drug in distilled water, Carbopol 940 was added in the quantity of 0.3, 0.4, 0.5, and w/v. This solution was then stirred until Carbopol940 completely swells in it. After the complete swelling of Carbopol, Xanthan gum was added in the quantity 0.15, 0.20, 0.25%w/v. After the complete hydration of both the polymers PEG 400(10%) and Methylparaben (0.033%) were added to it. This resulting formulation was then kept at 40°C overnight until clear gel is obtained.

Formulation optimization[12]

32 full factorial design was applied to the formulation that showed the satisfactory results to see the effect ofvarying the concentration of variables Carbopol 940 (X1) and Xanthan gum (X2) on various responses like % cumulative drug release, viscosity, mucoadhesive strength. For the Carbopol 940 lower levels was 0.3 mg, middle was 0. 4mg and higher level was 0.5mg. Similarly for the Xanthan gum lower level was 0.15mg, middle was 0.20 mg and higher level was 0.25mg. Composition of all the batches is shown in table 1.

Table 1: Composition of Formulation

Composition → formulation code↓	Venlafaxine HCl (%w/v)	Carbopol 940(%w/v)	Xanthan gum (%w/v)	PEG 400 (%v/v)	Methyl paraben (%v/v)	Distilled water Upto (ml)
F1	2.5	0.3	0.15	10	0.033	100
F2	2.5	0.4	0.15	10	0.033	100
F3	2.5	0.5	0.15	10	0.033	100
F4	2.5	0.3	0.20	10	0.033	100
F5	2.5	0.4	0.20	10	0.033	100
F6	2.5	0.5	0.20	10	0.033	100
F7	2.5	0.3	0.25	10	0.033	100
F8	2.5	0.4	0.25	10	0.033	100
F9	2.5	0.5	0.25	10	0.033	100

Evaluation of in situ nasal gel

1] pH[13]

pH of each formulation was determined by using Digital pH meter (systronics digital pH meter 335).The pH meter was calibrated using pH 4 and pH 7 buffer by using standard buffer tablet.

2]Viscosity [14]

Viscosity (rheological properties of prepared gel was determined with the help of Brookfield Viscometer; type DV-II+PRO using spindle no-62 and 63.Viscosity of formulations were determined at two different pH, formulation pH and at pH 7.4 with varying shear rate.

3] Measurement of gel strength [15]

A sample of 50g of the nasal gel was put in a 50 ml graduated cylinder. A weight of 5 g was placed onto the gel surface. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm deep into the gel.

4]Mucoadhesive force (detachment stress) [16, 17]

The mucoadhesive strength of each formulation was determined by measuring the force required to detach the formulation from goat nasal mucosal tissue by using a modified bioadhesion test apparatus that is modified physical balance. *In vitro* mucoadhesion studies were conducted using modified bioadhesion test assembly described by Mohammad et.al

Fabrication of equipment: The equipment was fabricated by us in the laboratory as shown in figure 1. A double beam physical balance was taken, both the pans were removed. The left pan was replaced with a brass wire, to which was hanged a Teflon block (A), also locally fabricated. The dimensions are a Teflon block of 3.8 cm diameter and 2 cm height was fabricated with an upward position of 2 cm height and 1.5 cm diameter on one side. The right pan (C) was replaced with a lighter pan so that, the left pan weighs 5.20gm more than the right pan. The lower Teflon block was intended to hold the mucosal tissue (D) of goat nasal mucosa and to be placed in a beaker containing simulated nasal solution pH 6.4. (E).

Measurement of adhesion force

Goat nasal mucosa was obtained commercially; the nasal mucosa was collected into a sterile container containing sterile buffer solution of pH 6.7. The nasal mucosa brought was stored in a refrigerator until use. The following procedure was used for all the test formulations using the above equipment. The nasal mucosa was removed from refrigerator and allowed to

attain equilibrium with ambient conditions in the laboratory. The goat nasal mucosa was carefully excised, without removing connective and adipose tissue and washed with simulated nasal solution. The tissue was stored in fresh simulated nasal solution. Immediately afterwards the membrane was placed over the surface of lower Teflon block (B) and secured. This assembly was placed into beaker containing simulated nasal solution pH 6.4 at 37 ± 20 ○ C. From each batch, some quantity of gel was taken and applied on the lower surface of the upper Teflon block. The beaker containing mucosal tissue secured upon lower cylinder (B), was manipulated over the base of the balance so that, the mucosal tissue is exactly below the upper cylinder (A). The exposed part of the gel was wetted with a drop of simulated nasal solution, and then a weight of 10 gm was placed above the expanded cap, left for 10 minutes. After which the gel binds with mucin. The weight was removed. Then slowly and gradually weights were added on the right side pan till the gel separates from the mucosal surface/ membrane. The weight required for complete detachment is noted (W1) (W1-5.20gm)) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

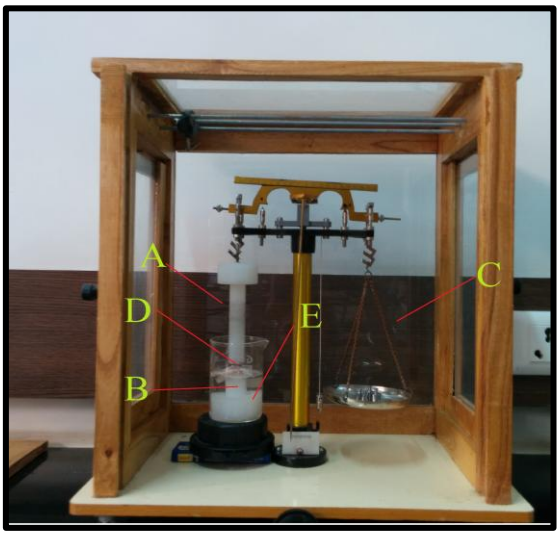

Fig 1: Modified mucoadhesion test apparatus (Fabricated)

5] Drug content [18]

Drug content was determined by taking 1ml of formulation was taken in 100 ml volumetric flask. It was dissolved in distilled water properly and final volume was made to 100 ml with distilled water. 1ml quantity from this solution was transferred into the 10ml volumetric flask and final volume was made to 10ml by using distilled water Finally the absorbance of prepared solution was measured at 224nm by using UV visible spectrophotometer. By using absorbance value % drug content in the formulation was calculated.

6] In Vitro Drug Release study [19]

A)Preparation of simulated nasal solution: Weigh accurately 0.87% NaCl, 0.31%KCl and 0.088% CaCl2·2H2O and dissolve in 1000 ml of distilled water to produce simulated nasal solution; finally adjusted the pH with phosphoric acid to 6.4. [20]

B]In vitro release study of the formulation was carried out using laboratory designed diffusion cell through egg membrane. From the gel 1 ml was placed in donor compartment and freshly prepared simulated nasal solution (The simulated nasal fluid (SNF) contained in receptor compartment (100ml)). Egg membrane was mounted between donor and receptor compartment. Temperature of receiver compartment was maintained at 37 ± 2^0C during experiment and content of the receiver compartment was stirred using magnetic stirrer. The position of donor compartment was adjusted so that egg membrane just touches the diffusion fluid. An aliquot of 1 ml was withdrawn from receiver compartment after 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hrs. and same volume of fresh medium was replaced. Aliquot so withdrawn were suitably diluted and analyzed using UV visible spectrophotometer at 223 nm. The concentration of drug was determined from a previously constructed calibration curve.

(y = 0.0464x + 0.0127, R2 =0.998).

7] Drug release kinetics [21]

It is generally understood that the release of the drug from gels can be considered as mass transport phenomenon involving diffusion of the molecules from a region of higher concentration to a region of lower concentration in the surrounding environment. The in vitro drug release data was fitted to different models, i.e. zero order, first order, Higuchi and Connor's and Korsemeyer's Peppas to study the drug release mechanism of the formulation.

8]*In Vitro* permeation study [22]

Natural membranes are utilized to determine in vitro permeation study to mimic the in vivo permeation patterns. In this experiment goat nasal mucosa was utilized because the respiratory area of goat is large and it is easy to get. Fresh mucosal tissue was removed from the nasal cavity of goat. The tissue was placed on the diffusion cell with permeation area 0.75cm2. The acceptor chamber of the diffusion cell (laboratory designed) with a volume capacity 100ml was filled with simulated nasal fluid (SNF) contained accurately 0.87%NaCl, 0.31% KCl and 0.088%CaCl2·2H2O. From the gel formulation 1ml (25mg equivalent) ml of was placed in donor compartment .At predetermined time point of 30 min, 1,2,3,4,5,6,7 and 8 hrs 1ml of sample was withdrawn from the acceptor compartment replacing the sample

removed with simulated nasal fluid after each sampling for period of 8 hrs .Then samples were specifically diluted and absorbance was noted at 223nm. Permeability coefficient (p) was calculated by the following formula:

$$P = \frac{dQ/dt}{C0.A}$$

Where, dQ/dt is the flux or permeability rate (mg/h), C0 is the initial concentration in the donor compartment, and A is the effective surface area of nasal mucosa.

9] Accelerated stability study[23]

Stability studies were conducted according to ICH guidelines $40^0C \pm 2^0C/75\% \pm 5\%$ RH to test the physical and chemical stability of the developed in situ nasal gel. A sufficient quantity of pH sensitive in situ gel, in screw capped vials was stored at different stability condition.

RESULTS AND DISCUSSION

1] Compatibility study

a) Infrared Spectroscopy

IR spectra of Venlafaxine Hydrochloride, polymers and physical mixture were generated. The IR absorption bands observed in the IR spectrum of drug and polymers resembles with that of found in the physical mixture proves compatibility of drug with polymers.

b) Differential scanning calorimetric analysis

DSC thermogram of drug shows strong endothermic peak at 217.09^0C and physical mixture exhibited characteristic peak at 210.24^0C. From the results it can be concluded that there is no interaction between drug and polymers because there is no significant shifting of peaks.

2] pH

The normal physiological pH of the nasal mucosa ranges from 4.5-6.5. But the nasal cavity has the capability to tolerate pH between 3-10. pH of all formulations was found to be between 5.2 to 5.8 that is within the range, which are presented in the Table 2.

3] Viscosity

Viscosities of all the formulations were noted at formulation pH and pH 7.4. It was observed that as the pH increases viscosity also increases. Mucoadhesive polymer Xanthan gum is also having synergistic effect with pH .All the formulations showed psudoplastic flow. Viscosities of all formulations at 25 rpm are shown in table 2 shows viscosity profile of all formulations.

4] Gel strength

Gel strength was recorded for all the formulations by using laboratory designed apparatus. It was observed that gel strength is showing synergistic effect with the viscosity, as the polymer concentration and pH increasesgel strength also increases. Gel strength for theformulations is noted in Table 2.

5] Drug content

Drug content found in the in situ nasal gel formulations resembled that of literature value. Range of drug content was 99-101%. Therefore uniformity of content was maintained in all formulation. Drug content of all the formulations is listed in Table 2.

6] Mucoadhesive strength

Mucoadhesive strength was determined by measuring the force required to detach the formulation from mucosal surface that is detachment stress. Results reveal that increase in Carbopol 940 and Xanthan gum concentration increases the mucoadhesive strength. This was due to interaction of polymeric chains with the mucin strands to form weak chemical bonds due to stronger mucoadhesive force. Mucoadhesive strength is listed in Table 2

7]*In vitro* drug release study

Out of nine formulations maximum release after 8 hrs was found for F5 formulation. This indicates release of 96.47% drug available for antidepressant activity of the drug. F5 formulation showed steady state release up to 8hrs which also indicates that this formulation would show better contact with biological membrane. Drug release of all the formulations is listed in Table 2.*In-vitro* drug release profile of all formulations shown in (Fig.2).

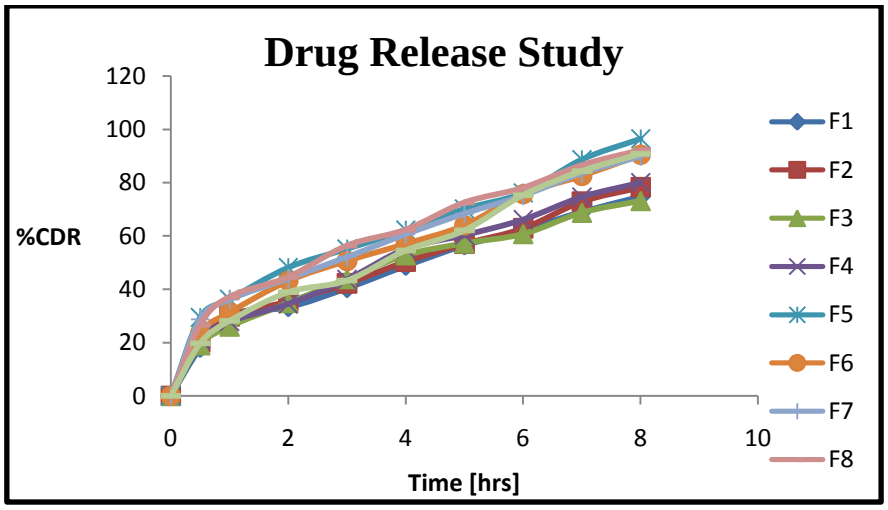

Fig. 2: *In-vitro* drug release profile of all formulations

Table 2: Evaluation parameters for all the formulations

Formulation code	pH	Gel strength [sec]	Viscosity [cps] at 25rpm	Drug content [%]	Mucoadhesive strength [gm]	In-vitro drug release study
F1	5.66±0.01	1.05±0.015	158.4	99.95 ±0.00042	22.4 ±0.14	74.76±0.0002
F2	5.47±0.015	1.14±0.012	302.6	99.66±0.00067	27.57±0.036	78.25±0.00007
F3	5.22±0.026	1.22±0.02	589.1	100.29±0.01	43.93±0.025	73.17±0.0002
F4	5.51±0.022	1.22±0.01	587.2	100.71±0.00049	59.66±0.05	80.00±0.0001
F5	5.36±0.022	1.24±0.021	563.2	99.60±0.01	70.15±0.026	96.47±0.0002
F6	5.45±0.017	1.27±0.022	869.3	100.56±0.00007	96.87±0.021	90.47±0.00007
F7	5.68±0.012	1.38±0.021	599.3	99.65±0.00024	69.97±0.032	88.85±0.0001
F8	5.81±0.01	1.42±0.031	722.3	101.13±0.00081	107.73±0	92.20±0.0002
F9	5.73±0.014	1.66±0.026	1417.5	100.46±0.0005	145.02±0.025	90.86±0.0001

8] Drug release kinetics

The classical zero order release curve was found to be linear ($r^2 = \geq 0.90$). The curves plotted according to first order and Higuchi and Conner's were also found to be linear. ($r^2 = \geq$ 0.90 for first order and 0.90 for Higuchi and Connor's data) respectively. For the Korsemeyer's Peppas release curves r^2 was found to be ≥ 0.90 for all 9 formulations and n value was found to be nearto 0.5 which indicates that all the formulations show fickian release.The drug release occurs probably by swelling and erosion and dissolution.

9]*In- vitro* permeation study

In vitro drug release was observed for the optimized formulation by using goat nasal mucosa. Permeation of the drug from goat nasal mucosa was studied for 8 hrs. It was found to be 86.52% at 8th hr. Permeation of the drug shows synergistic mechanism with that of in vitro drug release.Permeation study of the optimized formulation is listed in Table 3.*In-vitro drug* permeation profile of optimized formulation shown in (Fig.3).

Table 3: *Ex-vivo*permeation study for optimized batch F5.

Sr no.	Time (hrs.)	Drug permeation rate (mg/cm/hr.) (± S.D.)	% Cumulative drug permeation (±S.D.)
1	0.5	0.4672 ±0.026	17.53±0.094
2	1.00	0.4096±0.014	32.52±0.041
3	2.00	0.2357 ±0.032	40.22±0.11
4	3.00	0.1827±0.01	49.53±0.051
5	4.00	0.1437±0.015	55.64±0.085
6	5.00	0.1261±0.02	64.14±0.1053
7	6.00	0.1167±0.014	69.74±0.09
8	7.00	0.1020±0.026	80.4±0.15
9	8.00	0.090±0.03	86.52±0.17

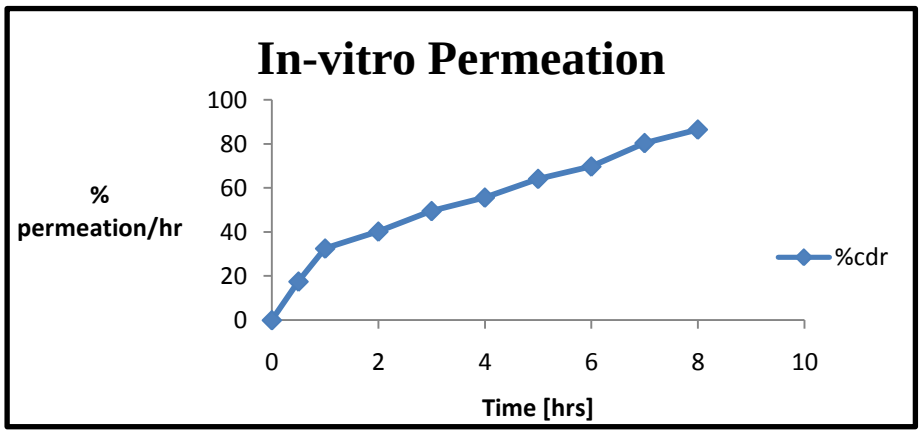

Fig 3: *In- vitro* permeation study for optimized batch F5

10] Accelerated stability study

Results of the stability studies showed that there is no change in the physical parameters of the formulation. Drug content of the formulation was also found to be same as that before stability testing.

Statistical analysis

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of Carbopol 940 (X1) and Xanthan gum (X2) were selected as independent variables and the dependent variable was% drug release, viscosity and Mucoadhesive strength. The data obtained were treated using Design expert version 7.0.software and analyzed statistically using analysis of variance (ANOVA). The data were

also subjected to 3-D response surface methodology to study the interaction of Carbopol940 (X1) and Xanthan gum(X2) on dependent variable. ANOVA for the dependent variable % drug release. The values of X1 and X2 were found to be significant at p <0.05, hence confirmed the significant effect of both the variables on the selected responses. From this data optimum concentration of Carbopol 940 0.4%w/v and Xanthan gum 0.2%w/v was found.3-D response surface Shown in (Fig.4), (Fig.5) and (Fig.6).

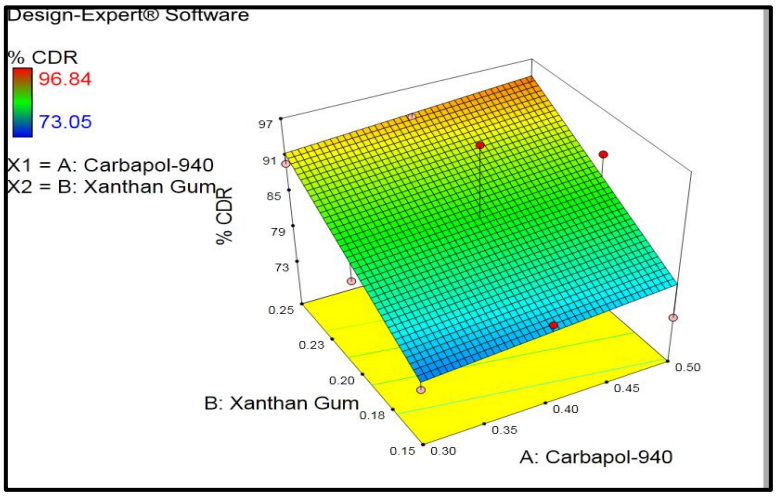

Fig.4: Surface response plot showing effect of Carbopol 940 and xanthan gum on drug release.

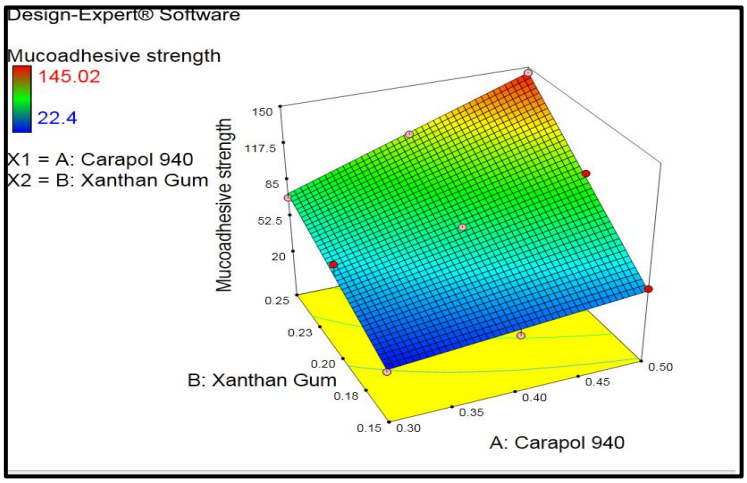

Fig.5: Surface response plot showing effect of Carbopol940 and xanthan gum on Mucoadhesive strength

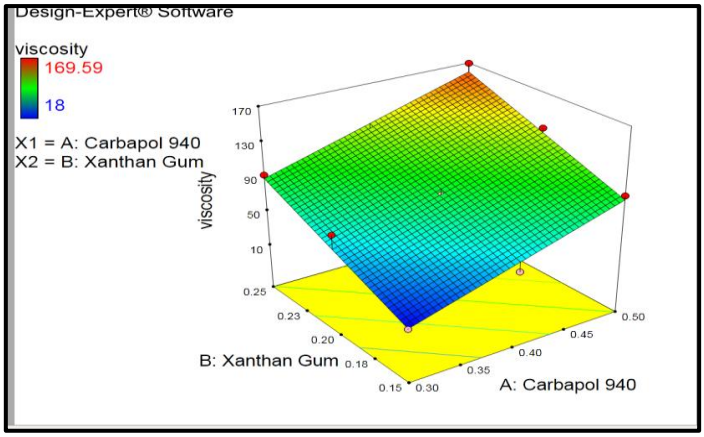

Fig.6: Surface response plot showing effect of Carbopol 940 and xanthan gum on Viscosity.

Optimized formula

After generating model equations relating main effects and responses, various gel formulations containing Venlafaxine Hydrochloride were optimized based on in vitro drug release (Y1) at 8hrs, Viscosity (Y2), and mucoadhesive strength (Y3). The optimal values for responses were obtained by numerical analysis based on the criteria of desirability, and optimal batch was selected. Optimized batch [F5] was having highest drug release, optimal viscosity andmucoadhesive strength. This reveals that mathematicalmodel obtained by factorial design to produce optimized responses was well fitted.

CONCLUSION

The formulation and development of*in situ mucoadhesive* gelling system for nasal administration for an antidepressant drug Venlafaxine Hydrochloride by using Carbopol 940 and Xanthan gum achieves the systemic delivery of drug through the nasal route and thereby reducing the dose of drug, avoiding its first pass metabolism. The in situ gels so prepared were characterized for its gelation properties, viscosity, gel strength, mucoadhesion, drug content, drug release rate. The results of a 3^2fullfactorial design revealed that the amount of Xanthan gum and Carbopol 940 significantly affect the dependent variables such as % cumulative drug release, mucoadhesive strength, viscosity.Thus, it can be concluded that, by adopting a systematic formulation approach, an optimum point can be reached in the shortest time with minimum efforts.

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