



FORMULATION AND EVALUATION OF NASAL IN-SITU GEL OF ESCITALOPRAM OXALATE FOR TREATMENT OF DEPRESSION

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ABSTRACT

The purpose of present study was to deal with develop intranasal drug delivery system of Escitalopram oxalate using thermosensitive polymer (Poloxamer -F188) and mucoadhesive polymer (Carbopol – 934P) *In-situ* forming polymeric formulation drug delivery system is in sol form before administration in the body, but once administered, that undergoes gelation in -situ to form a gel. Formulations were modulated so as to have gelation temperature below 34°C to ensure gelation at physiological temperature after intranasal administration. 30% Pluronic F68 was used and to it different concentrations of Carbopol -934P were added (0.1 to 0.6%). The bioavailability of a drug and its therapeutic effectiveness are often influenced by route of administration. The drug delivery system through nasal mucosa is a popular novel drug delivery method because mucous membranes of nasal cavity are relatively highly permeable, allowing for the rapid uptake of a drug into the localized and systemic circulation and to avoid the first pass metabolism. Many therapeutic drugs are difficult to reach the central nervous system (CNS) from the systemic blood circulation because the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier form a very effective barrier which prevents most molecules from passing through. The prepared Insitu gel was prepared by Cold Technique and then various evaluation were done such as desirable pH, gelation forming temperature, gel melting temperature, effect of rpm in viscosity and temperature and finally in-vitro diffusion study other tests were performed to get the desired formulation which achieves for the intranasal delivery. The results revealed that as the concentration of Carbopol increases there was a decrease in the gelation temperature. pH of all the formulations were found to be within the range between 5.1-6.4 and the nasal mucosa can tolerate the above mentioned pH of the formulations. Viscosity of all the six formulation was measured in Fungilab viscometer at different RPM (12, 10, 8, 6, 4 and 2) in table no.5.12-5.16 the effect of RPM on Escitalopram in-situ gel was observed it shows that as the RPM increase the viscosity also increased while the torque was measured against the rpm. As the rpm decreased the torque value is also decreased so, gel is converts from Newtonian to non- Newtonian behavior. The Kinetic studies of all the formulation 'r' value of Zero order kinetic were the ranges from 0.950-0.991 to; similarly the 'r' First order kinetic were ranges from 0.965-0.976; Huguchi matrix 'r' range from 0.982 – 0.950; Kosmeyer peepes 'n' 0.988 – 0.950. It is found that formulation F1 which is containing Carbopol-934P (0.1%) shows 94.05% drug releases (shows the highest release) in 4hrs. Hence formulation F1 was found to be most optimized formulation. From the kinetic models it is observed that the Huguchi model is dominant for the release of Escitalopram oxalate from in-situ gel and the mechanism of release is non-fickian. Intranasal drug delivery through nasal mucosa has been reported as an emerging tool to improve bioavailability of many CNS drugs. The biopolymers used and their compositions in the in-situ gels preparation greatly affected the drug release which allows absorption in the nasal mucosa.

KEYWORDS: Nasal in-situ gel, Poloxamer F188, Carbopol-934P, Escitalopram oxalate.

INTRODUCTION

Intranasal administration has been practice for thousands of years. Nasal therapy has been recognized from treatment in Ayurvedic System of Indian medicine, it is called “NASAYA KARMA”. In recent years many drugs have been shown to achieve better systemic circulation bioavailability through nasal than by oral administration. In addition intranasal drug delivery enable dose reduction, rapid attainment of therapeutic

blood levels, quicker onset of pharmacological activity and fewer side-effects. Nasal area of drug delivery received additional attention with a timely seminar organized by Dr. Y.E. Chien in 1984 the seminar entitled “**Intranasal Drug Administration for Systemic Medications**” was instrumental in placing nasally administered medications at the fore front of drug delivery.

Diseases of the Central Nervous System (CNS) such as depression, schizophrenia, meningitis, migraine, Parkinson's disease and Alzheimer's disease require delivery of the drug to the brain for treatment. However such transport remains problematic, especially for hydrophilic drugs and large molecular weight drugs, due to the impervious nature of the endothelial membrane separating the central interstitial fluid, the Blood-Brain Barrier (BBB).

Escitalopram oxalate is the pure (S) enantiomer of racemic citalopram and is a selective serotonin reuptake inhibitor (SSRI). Escitalopram is used in the treatment of depression and anxiety. It is approved for the treatment of major depressive disorder and generalized anxiety disorder; other indications include social anxiety disorder, panic disorder and obsessive compulsive disorder. Escitalopram oxalate acts by increasing intrasynaptic levels of the neurotransmitter serotonin by blocking the reuptake of the neurotransmitter into the neuron. Its half life is about 27-30 hours. It is metabolized in the liver, especially by the CYP3A4 and CYP2C19 after oral administration. Its bioavailability is 80% and protein binding is approximately 56%. It is poorly soluble in water so its absorption is less. It is acidic and its pKa value is 4.19 which is satisfactory for selection of drug. The Log Partition coefficient value is 3.45. The t_{max} value of Escitalopram oxalate is 4hr after multiple dosing. The dose of 5-20mg once a day and is used for treatment of depression.

In-situ gels are the novel drug delivery systems that favors the ease and convenience of administration and delivery of accurate dosage forms which are the major problems encountered by the normal semi solid dosage forms. An in situ mucoadhesive gel appears to be very attractive since they are fluid like prior to administration which makes them easy to administer as a drop allowing accurate dosing. Formulation of in situ gels depends on various physical and chemical stimuli.

Poloxamers or Pluronics are a class of thermo reversible gels that have the capacity to make, break and modify the bonds responsible for holding the network together. Their thermo reversible property make them useful as a carrier for most routes of administration including oral, topical, intranasal, vaginal, rectal, ocular and parenteral routes. Reverse thermal gelation and low toxicity have been the basis of research into the use of Pluronics as a possible drug delivery system in man.

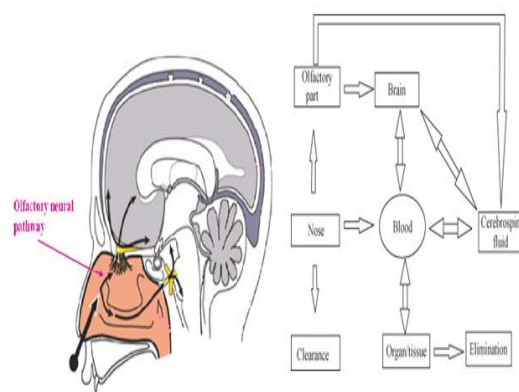


Fig. 1(a). Position of olfactory bulb with respect to brain and nasal cavity.

Fig. 1(b). Different pathways for reaching the brain after intranasal administration.

Figure no. 1- Nose to Brain Drug Delivery System

MATERIAL AND METHOD

Materials

Escitalopram oxalate was gifted sample from WINDLAS pharmaceuticals Pvt. Ltd. All materials such as Poloxamer F188, Carbopol- 934P, Propylene glycol and Benzalkonium chloride used in these studies were purchased from Central Drug House Ltd, New Delhi (IND).

Methods

Preformulation studies

Preformulation is an exploratory activity that begins early in drug development. Preformulation studies are designed to determine the compatibility of initial excipients with the active substance for a biopharmaceutical, physicochemical, and analytical investigation in support of promising experimental formulations. Data from preformulation studies provide the necessary groundwork for formulation attempts. The selected drugs and excipients were standardized as per respective pharmacopoeial specifications, wherever applicable. Excipients which were not official in Pharmacopoeias were standardized as per the manufacturers' specifications. The certificates of analysis provided by the suppliers in case of gift samples have been duly included in the appropriate sections.

Identification of drug

➤ Appearance

Drug powder was inspecting visually for color and form of drug.

➤ Determination of pH

The pH of 1% w/v concentration of pure drug in water was tested by using calibrated.

➤ Solubility

The solubility of was tested in various solvents such as methanol, distilled water and phosphate buffer pH6.4.

➤ Determination of melting point

The sample was loaded in to sealed capillary (melting point capillary) which was then placed in melting point

apparatus. The sample was then heated and as the temperature increase the sample was observed to detect the phase change from solid to liquid phase. The temperature at which the phase changes occur gives the melting point.



Figure no.2 - Determination of Melting Point

Drug excipients compatibility study

Through FTIR- Pure drug matched with that of FTIR spectra of drug and polymer.

Fourier transform infra red (F.T.I.R.)

- Infrared spectrum of Escitalopram oxalate was determined by using Fourier Transform.
- Infrared Spectrophotometer using KBr disks method. The sample (0.5 to 1.0 mg) is finely.
- Then grounded and intimately mixed with approximately 100 mg of dry potassium bromide powder.
- Grinding and mixing can be done with mortar and pestle. The mixture is then pressed into a transparent disk in an evaluable die at sufficiently high pressure.
- Suitable KBr disks or pellets can often be made using a simpler device such as a hydraulic press.
- The base line correction was done using dried potassium bromide. Then, the spectrum of dried mixture of drug and potassium bromide was scanned from 2000 cm^{-1} to 400 cm^{-1} .
- FTIR has been used to assess the interaction between drug and polymers.

Preparation of Calibration Curve

Instrument: UV- Spectrophotometer

- The absorption maximum (λ -max) for Escitalopram oxalate was determined by scanning the drug solution within the range of 200 nm to 400 nm using a UV- Visible Spectrophotometer. The drug exhibited a λ -max at 265nm.
- **In pH 6.4 buffer:** Preparation of phosphate buffer saline pH 6.4.

Dissolve 1.7 gm of disodium hydrogen phosphate, 1.36 gm of potassium dehydrogenate phosphate and 7.02 gm of NaCl then make up the volume upto 1000 ml in volumetric flask.

- 10mg of Escitalopram was weighed accurately and dissolved in solvent. The volume of solution was made up to 100ml. The solution was marked as **stock solution**.

1) From Stock solution dilutions having concentration 1 $\mu\text{g/ml}$, 2 $\mu\text{g/ml}$, 4 $\mu\text{g/ml}$, 6 $\mu\text{g/ml}$, 8 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, 12 $\mu\text{g/ml}$, 14 $\mu\text{g/ml}$, 16 $\mu\text{g/ml}$, 18 $\mu\text{g/ml}$ and 20 $\mu\text{g/ml}$ were prepared.

2) Above prepared solutions were observed in double beam UV-Spectrophotometer to measure the absorbance, in increasing order of concentrations.

Preparation of Nasal In-situ gel

There are two methods for the preparation of in situ gel. Besides of all two methods cold process is generally used for the preparation of in situ gel and usually preferred. In the case of hot process it required lengthy procedure and lump and forth and other type of impurities may occur.

Cold process

For the preparation of in-situ nasal gel the **Cold technique** was selected. The technique was as follows: Accurate quantity of Propylene glycol was mixed with definite volume of water. To this mixture add specified amount of Polymers (Poloxamer and Carbopol). This mixture was stirred until clear solution was formed. The resulting solution was kept in refrigeration at 4°C overnight.

Hot process

Pluronic F-188 dissolved in water approximately at 70°C. Active substances that are insoluble in water are dissolved in Methanol, Isopropyl alcohol or Propylene glycol at 70°C and mixed with warm aqueous phase to form a homogeneous mass before addition. The gel forms when the solution cools to room temperature.

METHODOLOGY

The formulations were prepared by using various concentrations of polymers which are thermosensitive and Bioadhesive in nature. The different polymers were dissolved in cold water by agitation at room temperature. Poloxamer was added into the ratio of water: propylene glycol with continuous agitation, then Carbopol and Benzalkonium chloride was added slowly with continuous agitation. The solution was kept in refrigerator for cooling upto 4°C. The resulting formulations were then kept overnight at 4°C until clear and transparent solution formed. Concentration varying from 15, 20, 30% w/v were screened preliminarily to decide possible concentration. Study the effect of different polymers concentration on gelation temperature, gelation time and gelatin capacity.

Table No. 1 Concentration of Polymer

S.No	Concentration of Poloxamer (% w/v)	Gelation temperature	Gelation time (Min.)	Gelation capacity
P1	15%	42 ⁰ C	2.14	++
P2	20%	40 ⁰ C	2.03	+++
P3	30%	41 ⁰ C	1.44	+++

+ gel forms after few minute, disperses rapidly; ++ immediate gelation, remains for few hours; +++ immediate gelation, remains for extended period of time.

Evaluation of prepared in-situ nasal gel

Clarity: The clarity may be determined by visual inspection under the black and white background.

Determination of gel forming temperature

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

Gel Melting Point

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

Determination of viscosity

Viscosity of different formulations was measured with the fungilab viscometer at different RPM 2, 4,6,8,10,12 and temperature, using spindle PD.

Spreadability study

The spreadability study was done by slip and drag characters tic. A 20cm of long glass slide was fixed in the stands. Weight 1g gel on the glass slide then keep the gel between this slide and another glass slide having the same dimensions. A weight of 500g was placed on glass slides for 5min for the gel to spread on slide. The top glass plate was then subjected to pull with 50g of weight tied on the upper slide at a distance of 7cm. Lesser the time taken by the slides to move the specified distance of 7cm the better is the spreadability.

Spreadability is calculated from the following formula

$$S = M \cdot L / T$$

Where,

S= spreadability(g.cm/ sec)

M= wt on upper slide (g)

L= Length moved by the glass slide (cm)

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



pH of in situ nasal gel

It is known that the normal physiological pH of nasal mucosa is 5.5 – 6.5; however the nasal mucosa can tolerate solutions within pH range of 3-10.

In vitro diffusion study of prepared Escitalopram In-situ nasal gel

In-vitro release of Escitalopram oxalate from gel was measured through dialysis membrane done in Franz diffusion cell with maintained temperature 37.5°C at 50rpm, pH 6.4 was filled in receptor components. Specific amount of nasal gel was kept in donor compartment. Dialysis membrane was mounted on Franz Diffusion cell. Accurate amount of gel was placed about 5 mg of drug. At predetermined time 1ml of sample was pipette out and replenished immediately an equal amount of 6.4 phosphate buffer. Samples were diluted and absorbance was measured at 266 nm range.



Drug content

The prepared formulation take 1ml of smart gel and adjust to 10ml in volumetric flask and then dilute with 10ml of distilled water, 1ml from this solution again diluted with distilled water up to 10ml after this take absorbance of prepared solution at a particular wavelength of the drug by using U.V visible spectroscopy.

Drug content = absorbance X dilution factor / slope

Swelling Index

Prepared formulation nasal in-situ gel was weight 100 mg and was kept upon the aluminum foil, make some pores on the foil and put in-situ gel to it. Then take 50 ml of beaker add 6.4 phosphate buffers to it put aluminum foil containing gel upon the beaker. Then keep formulation for 12 hrs. After 12 hrs weight of swelling gel was calculated by equation:

Swelling Index = $W_1 - W_2 \times 100$

**Whereas, W_1 = initial wt of gel
 W_2 = wt. of swollen gel after 12 hrs**

Kinetic modeling

To determine the release pattern from designed micro beads loaded gel, the in vitro data was used and treated by various conventional mathematical models (Zero order, First order, Higuchi model and Korsmeyer-Peppas model). The value of R (correlation coefficient), k (release constant) and n (diffusion exponent) obtained from the curve fitting of release data were estimate a suitable release model for the formulation.

Zero order model

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

$$Q_0 - Q_t = K_0 t$$

Or $Q_t = Q_0 + K_0 t$

Where, Q_t is the amount of drug dissolved in time t.

Q_0 is the initial amount of drug in the solution.

K_0 is the zero order release constant expressed in units of concentration/time.

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

Applications

This model can be used to identify the dissolution of drugs from several types of modified release pharmaceutical dosage forms, transdermal system and also in matrix tablets with low soluble drugs in coated forms, osmotic systems, etc.

First order model

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

$$\log C = \log C_0 - Kt/2.303.$$

Where, C_0 is the initial concentration of the drug.

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

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

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

Applications – This model can be used to identify the drug dissolution in pharmaceutical dosage form for such drugs which are water soluble.

Higuchi model

The following equation is used to describe this model:

$$Q_t = K_H \times t^{1/2}$$

Where, K_H is the higuchi dissolution constant.

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

This is a hypothesis based model which is as follows

1. Initial drug concentration in the matrix is much higher than drug solubility.
2. Drug diffusion takes place only in one dimension.
3. Drug particles are much smaller than system thickness.
4. Matrix swelling and dissolution are negligible.
5. Drug diffusivity is constant.
6. Perfect sink conditions are always attained in the release environment.

Application

This model can be used to identify the dissolution of drugs from several types of modified release pharmaceutical dosage forms, transdermal system and also in matrix tablets with low soluble drugs.

Korsmeyer – Peppes model

The following equation is used to identify this model.

$$M_t/M_\infty = K t^n$$

Where, M_t/M_∞ is a fraction of drug released at time t.

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

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

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

Table no. 4.7 Interpretation of diffusion release mechanism from polymeric films.

Release exponent (n)	Drug transport mechanism
0.5	Fickian diffusion
$0.45 < n < 0.89$	Non fickian diffusion
0.89	Case II transport
Higher than 0.89	Super case II transport

RESULT AND DISCUSSION

Solubility

Escitalopram oxalate is soluble in methanol, sparingly soluble in water, slightly soluble in Ethyl acetate, insoluble in Heptane and freely soluble in 6.4 phosphate buffer solution as shown in Table No: 3.

Table no. 3 Solubility of Escitalopram oxalate

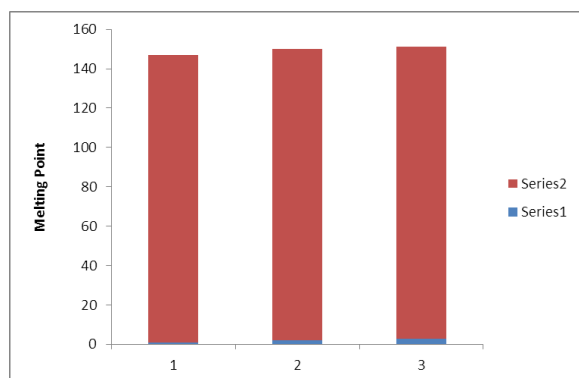
S.NO	Solvent	Descriptive term	Con.(µg/ml)	As per IP
1.	Water	Sparingly soluble	1.740	30-100
2.	Methanol	Soluble	3.311	10-30
3.	6.4 phosphate buffer	Freely soluble	3.954	1 -10

Melting Point

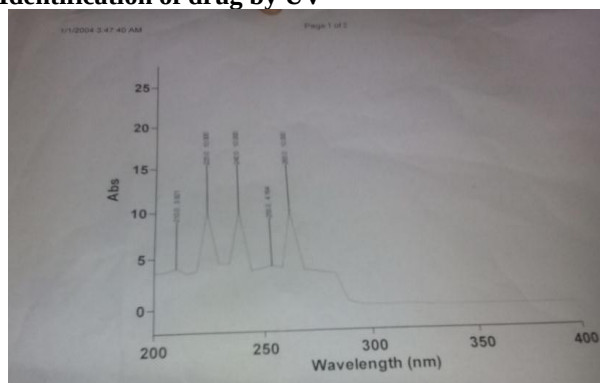
Melting point determination was done by capillary method.

Table no. 4 Melting Point of Escitalopram oxalate

S.NO	Observed Melting Point	Experimental value of melting point mean $\pm$ SD(n= 3)	Standard Range
1.	146	147 ± 1.154	$147-148^{\circ}\text{C}$
2.	148		
3.	148		

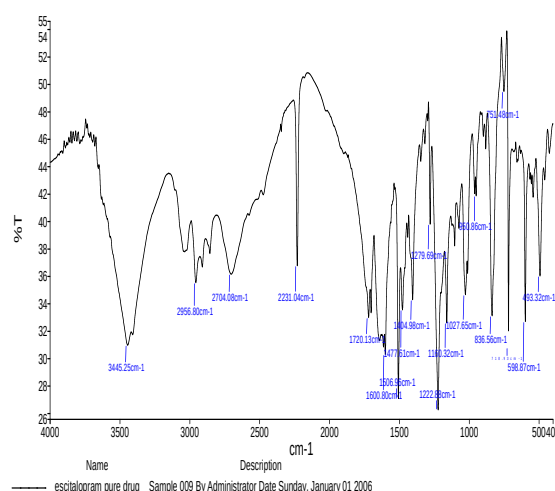
**Figure no.3 Bar diagram of Melting point**

Identification of drug by UV

**Figure no. 4 Escitalopram oxalate pure drug identification**

INFRARED SPECTROSCOPY

The IR spectrum of the pure Escitalopram oxalate sample recorded by FTIR spectrometer is shown in figure.

**Figure no.5 FTIR spectra of pure drug sample of Escitalopram oxalate**

Comparison of peaks between Drug and Excipients

The compatibility study was done by matching the peak of functional group of pure drug with drug and excipients mixture FTIR of pure drug and drug along with excipients where showed in fig. No significant changes can be seen in table no.5 in functional group of pure drug

and drug excipients mixture suggested that there is no interaction and it was observed that all the characteristics peaks of drug are present in the combination of drug and polymer spectra indicating the compatibility of drug with the polymer used.

Table no.5 Comparison peaks

S.No	Drug	PF-188	Carbopol(934)	Propylene Glycol	Standard peaks	Functional group	Stretching/Bending
1	3445.25	3448.09	3448.38	3333.7	3500-3100	N-H	Stretching
2	2956.8	2874.44	3045.97	2899.1	2900-2800	C-H	Stretching
3	2704.08	2744.04	2764.93	2726.6	2800-2700	C-H	Stretching
4	2231.04	2230.44	2231.56	2231.16	2500-2100	C=C	Stretching
5	1720.13	1641.11	1720.13	1738.02	1740-1720	C-O	-
6	1600.8	1600.76	1506.76	1649.88	1680-1600	C=C	-

UV Spectroscopy

Calibration curve was found to be that estimation of Escitalopram oxalate by UV Spectroscopy method at 265nm in 6.4 phosphate buffer solution. Standard calibration curve of Escitalopram shows the absorbance of standard solutions at different concentration 2-20 µg/ml. Data are shown in table. Figure shows a representative standard calibration curve with slope, regression coefficient respectively.

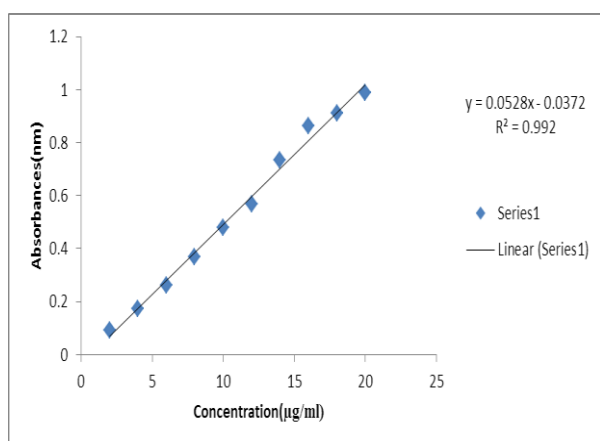


Figure no.6 Calibration curve of EO in phosphate buffer 6.4

Observed λ max of drug - 265nm.

Observed Beer's range of drug 2-20µg/ml.

Line of equation and r^2 value = ($y = 0.052x + 0.037$).

R^2 value = 0.992.

Table no.6 Final Composition table of In-situ Nasal gel:

Ingredients	F1	F2	F3	F4	F5	F6
Escitalopram oxalate	5g	5g	5g	5g	5g	5g
Poloxamer F188(w/v)	30%	30%	30%	30%	30%	30%
Carbopol(w/v)	0.1%	0.2%	0.3%	0.4%	0.5%	0.6%
Propylene glycol	20%	20%	20%	20%	20%	20%
Benzalkonium chloride	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Distilled water(ml)	100	100	100	100	100	100

Evaluation of Nasal in-situ gel



Clarity

The clarity of the all formulation found to be satisfactory and in normal vision condition the formulation was to be seen clear and transparent.

Table no.7 Result of Clarity

S.No	Formulation	Clarity
1	F1	Clear and transparent
2	F2	Clear and transparent
3	F3	Clear and transparent
4	F4	Clear and transparent
5	F5	Clear and transparent
6	F6	Clear and transparent

Determination of Gel forming Temperature, gel melting temperature and pH

Prepared in-situ gel evaluated for gelling forming temperature and gel melting point. At certain body temperature (25-27°C) solution phase is converted into gel phase due to addition thermosensitive Poloxamer 188 and mucoadhesive Carbopol-934P there is change in T_1 & T_2 gel formation can be seen in table no.5.10. Formulation F1, F2, F3, F4, F5 & F6 having gelling forming temperature 37, 33, 30, 28, 24 & 20. As a result the concentration Carbopol-934 is increase (0.1, 0.2, 0.3, 0.4, 0.5, 0.6) so, the gelation temperature decreases and gel melting temperature increase. The formulations containing concentration of Carbopol-934P higher than 0.5% were found to have very high viscosity and so were difficult to administer into the nostril.

Nasal cavity pH range is 5.5 – 6.5, but nasal mucosa has capacity to tolerate pH 3-10 also. The pH of all the formulation was measured by pH meter was found to be 6.4 – 5.1 in table no.8 as a result there is no significant change in the formulations. This natural pH shows non-irritant to nasal mucosa.

Table no. 8 Result of Gel forming, Gel melting temperature and pH

S.No	Formulation	Gel forming temperature (T_1)	Gel melting temperature (T_2)	pH
1	F1	37°C	49°C	6.4
2	F2	33°C	51°C	6.3
3	F3	30°C	68°C	5.4
4	F4	28°C	72°C	5.1
5	F5	24°C	83°C	5.3
6	F6	20°C	85°C	5.2

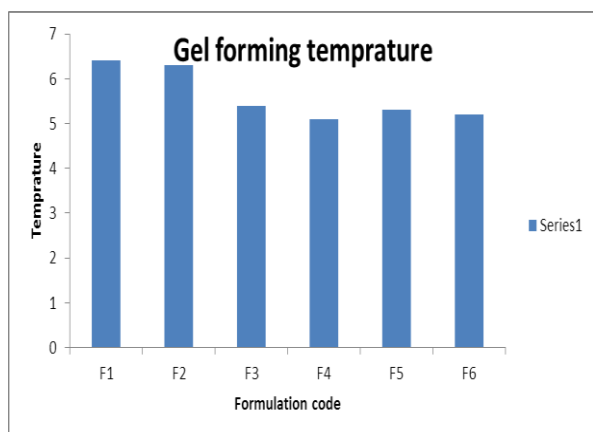


Figure no. 7 Gel forming temperature

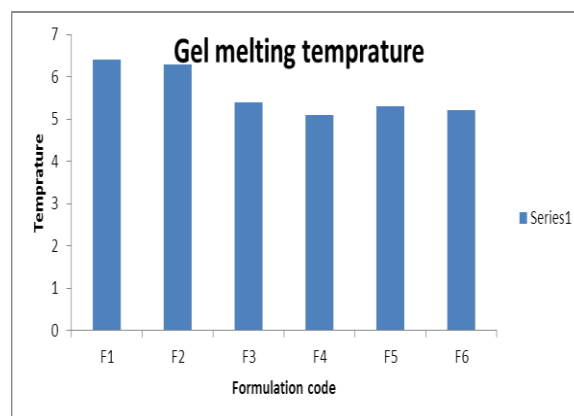


Figure no. 8 Gel melting temperature

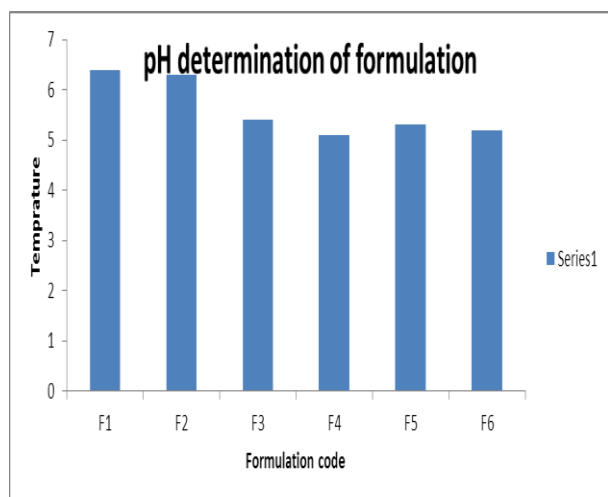


Figure no. 9 pH determination

Swelling Index: Swelling Index was found to be appropriate for nasal in-situ gel as per shown in table no. 9.

Table no. 9 Swelling Index of formulations (F1-F6)

S.No	Formulation	Swelling Index
1.	F1	80.64± 0.72
2.	F2	83.7±0.61
3.	F3	84.39±0.53
4.	F4	84.74±0.62
5.	F5	80.32±0.72
6.	F6	86.5±0.2

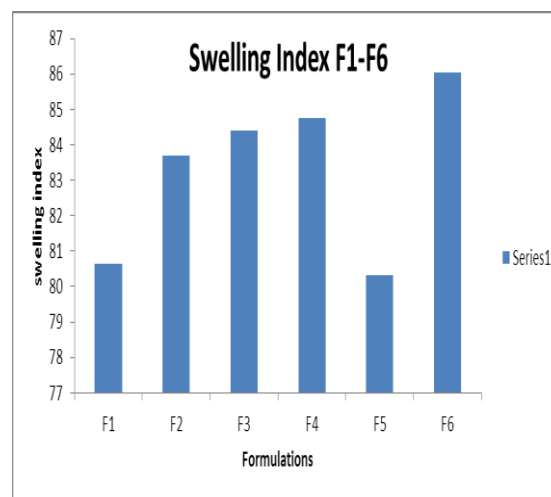


Figure no. 10 Graph b/w formulation and swelling index

Determination of Viscosity: The viscosity of formulations at 4°C temperature to 37°C is shown in Table 10 respectively.

Table no. 10 Viscosity formulations depending on temperature:

S.No	Temperature	F1	F2	F3	F4	F5	F6
1	4	529	741	783	827	884	937
2	6	631	835	851	876	895	986
3	8	853	993	962	985	991	1095
4	10	1063	1182	2189	2359	1032	1664
5	12	1175	2283	4715	3871	1493	2348
6	14	2769	3495	6982	4328	2849	3893
7	16	3194	6721	8914	5726	3287	4175
8	18	6592	7842	9474	7529	5384	6492
9	20	8392	8969	20836	22541	7421	8290
10	22	1016	1283	22859	24621	9274	18105
11	24	2044	2184	24981	25285	18591	19746
12	26	24829	25515	26943	27411	19952	20147
13	28	29729	27491	27741	28351	20159	21982
14	30	30191	31014	29306	29731	22971	27302
15	32	32823	33817	30632	32971	23143	26716
16	34	34010	35793	33921	34320	25718	26671
17	37	37856	36002	36286	35188	27152	26017

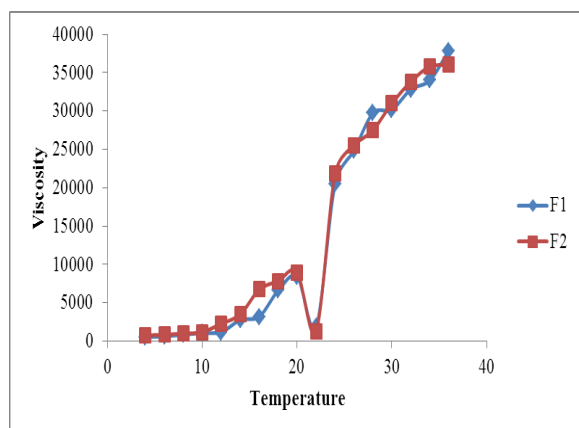


Figure 11 Graph b/w viscosity and temperature

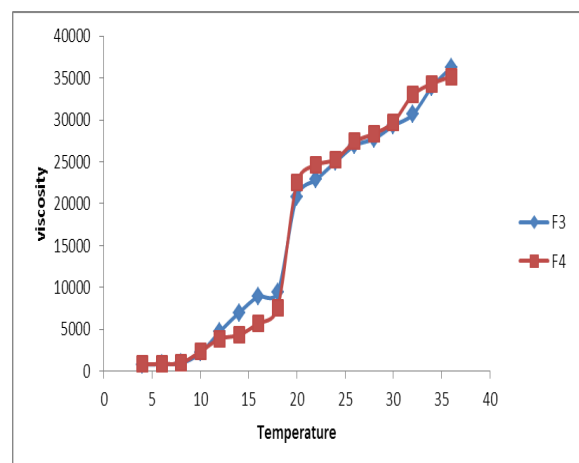


Figure no. 12 Graph b/w viscosity and temperature

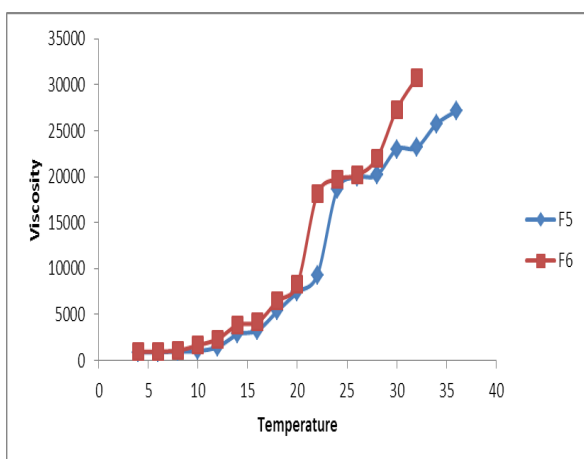


Figure no. 13 Graph b/w viscosity and temperature

• Table no.11 Viscosity of F1

Spindle	RPM	Torque	Viscosity(Cp)
PD	12	55.3%	6599.6
PD	10	54.7%	5299.6
PD	8	45.6%	1509.7
PD	6	32%	1423.3
PD	4	14.1%	866.5
PD	2	3.7%	349.8

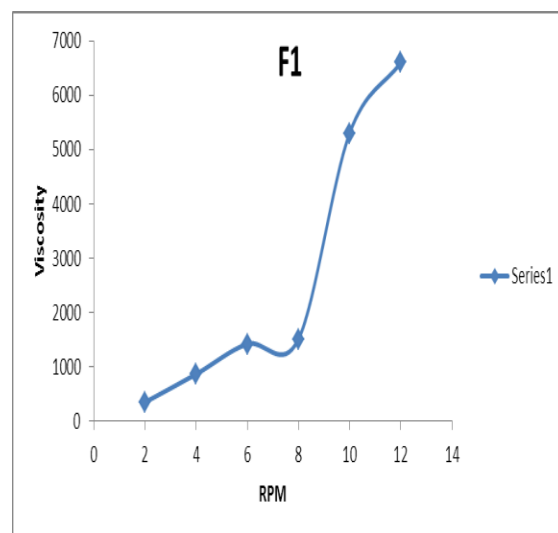


Figure 14 Graph b/w viscosity & RPM

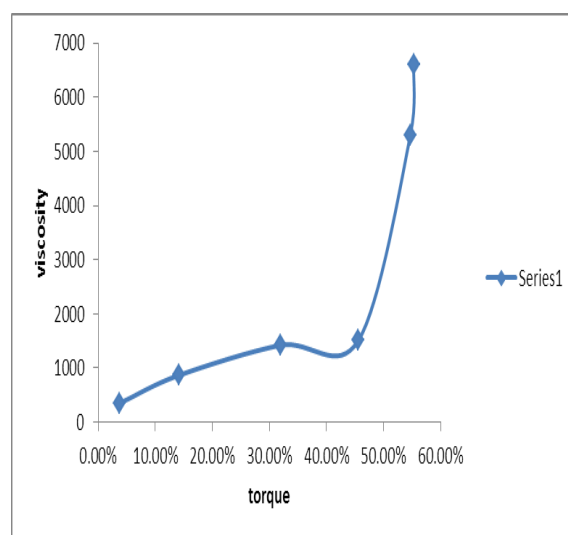


Figure 15 Graph b/w viscosity & torque

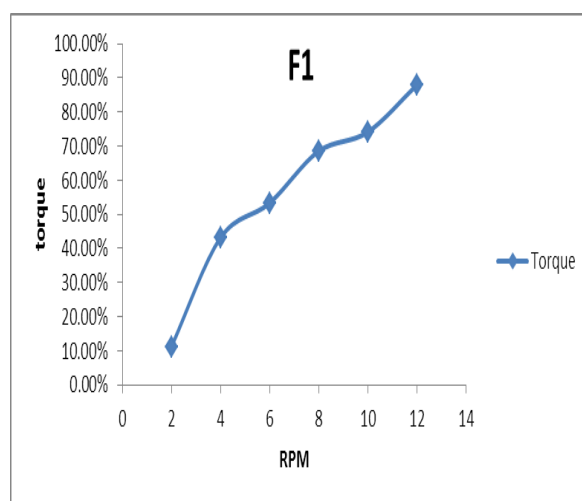


Fig no. 16 Graph b/w torque & RPM

- Table no.12 Viscosity of F2

Spindle	RPM	Torque	Viscosity(Cp)
PD	12	79.8%	9402.2
PD	10	72.1%	6768.3
PD	8	65%	2493.2
PD	6	55%	2178.9
PD	4	32.6%	1015.1
PD	2	30.1%	51.2

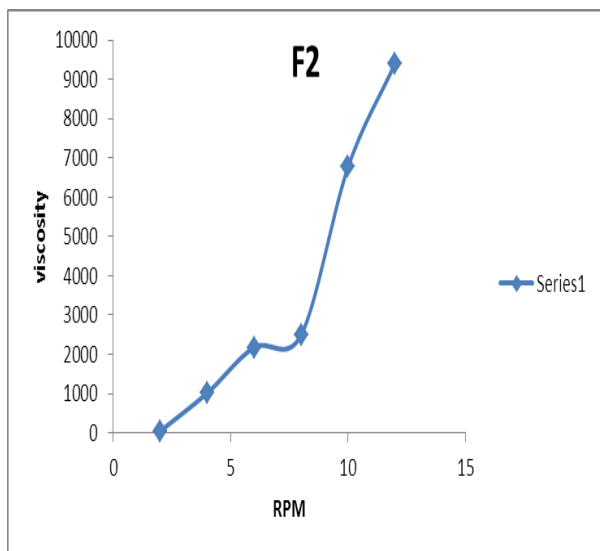


Figure 17 Graph b/w viscosity & RPM

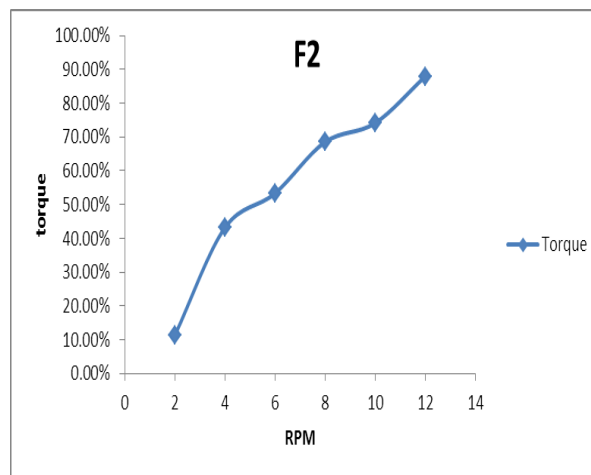


Fig 19 Graph b/w torque & RPM

- Table no.13 Viscosity of F3

Spindle	RPM	Torque	Viscosity(Cp)
PD	12	86.5%	9763.5
PD	10	76.4%	6752.5
PD	8	48.7%	5274.8
PD	6	25.01%	3580.1
PD	4	13.5%	2056.8
PD	2	9.4%	1028.4

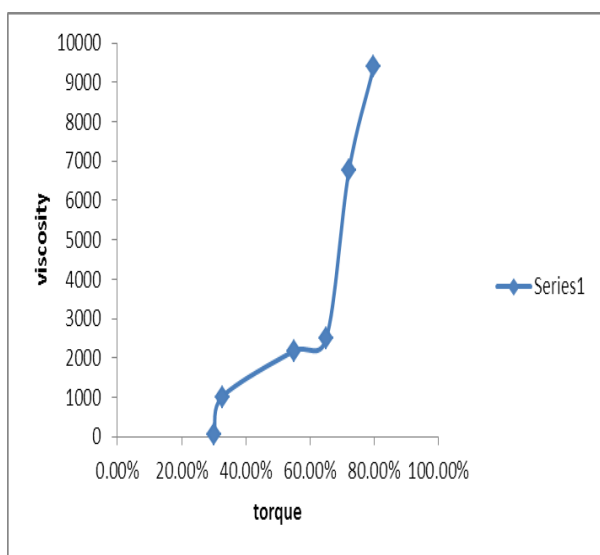


Figure 18 Graph b/w viscosity & torque

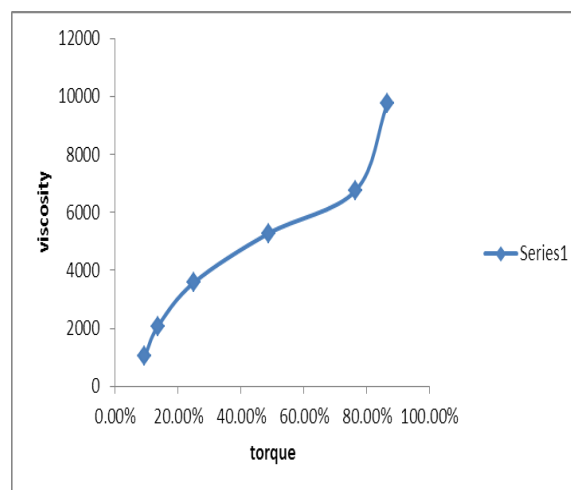


Figure 20 Graph b/w viscosity & torque

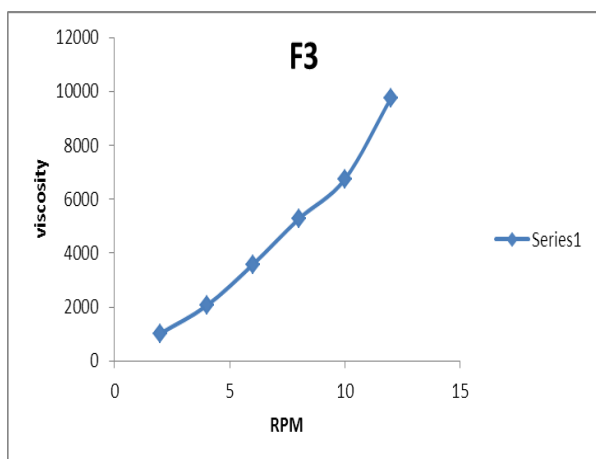


Figure 21 Graph b/w Viscosity & RPM

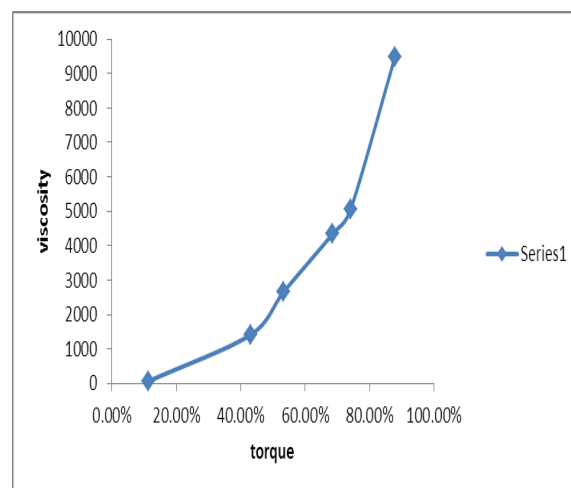


Figure 24 Graph b/w viscosity & torque

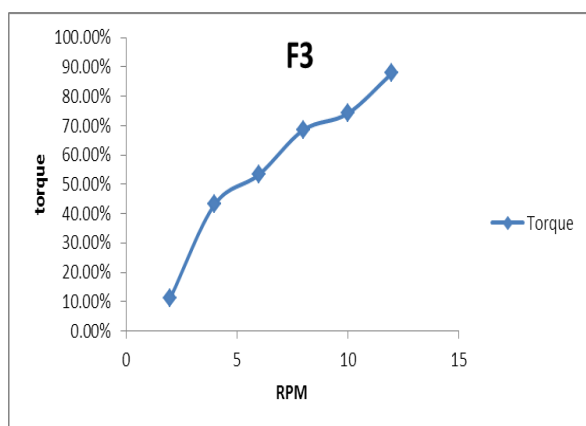


Fig. 22 Graph b/w torque & RPM

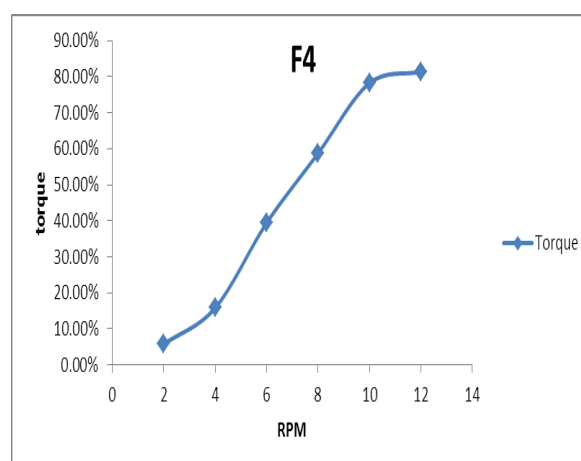


Fig. 25 Graph b/w torque & RPM

• **Table no. 14 Viscosity of F4**

Spindle	RPM	Torque	Viscosity(Cp)
PD	12	87.9%	655.26
PD	10	74.1%	5051.5
PD	8	68.5%	4341.8
PD	6	53.3%	2656.8
PD	4	43.1%	1417.5
PD	2	11.2%	61.1

• **Table no.15 Viscosity of F5**

Spindle	RPM	Torque	Viscosity(Cp)
PD	12	81.4%	7213.8
PD	10	78.2%	6839.5
PD	8	58.7%	5274.8
PD	6	39.4%	4408.3
PD	4	15.8%	3553.1
PD	2	5.8%	1026.1

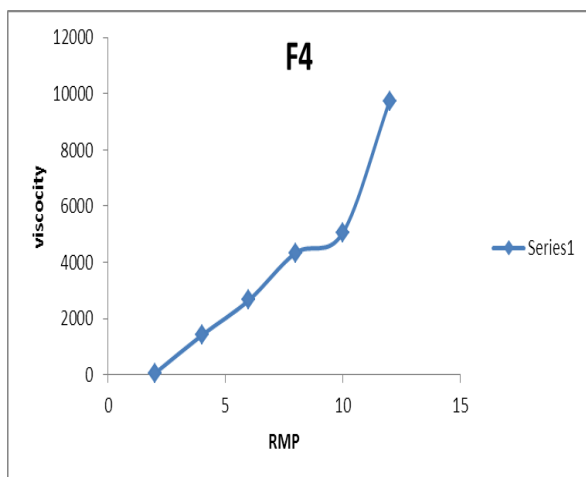


Figure 23 Graph b/w viscosity & RPM

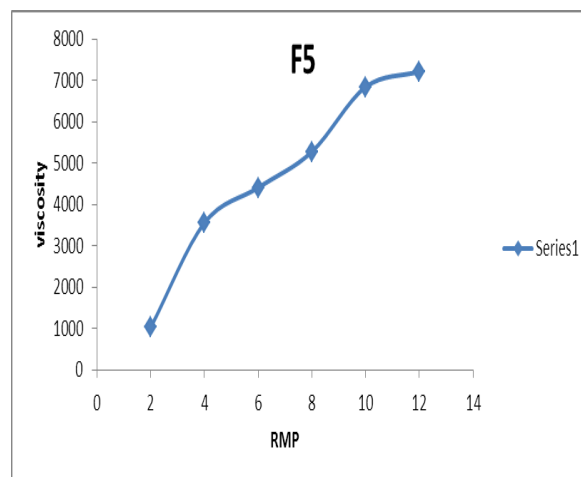


Figure 26 Graph b/w viscosity & RPM

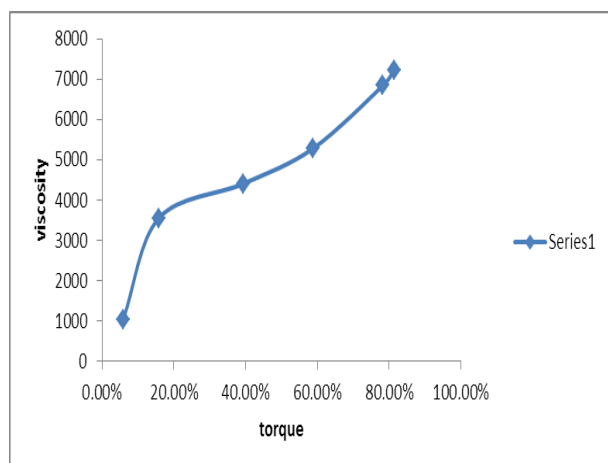


Figure 27 Graph b/w viscosity & torque

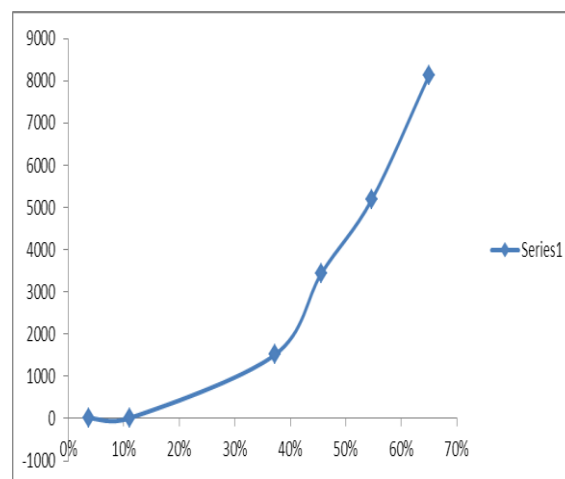


Figure 30 Graph b/w viscosity & torque

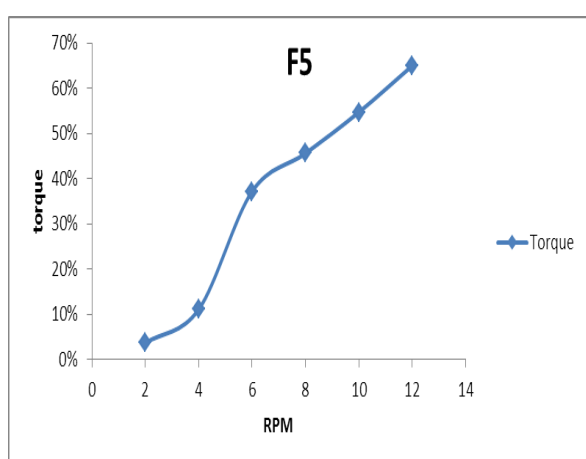


Fig. 28 Graph b/w torque & RPM

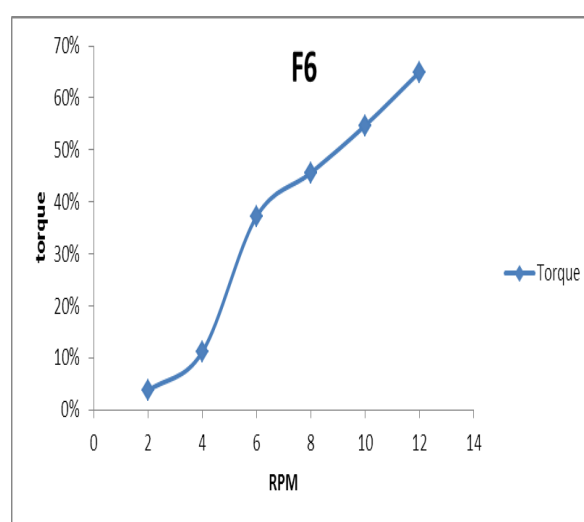


Fig. 31 Graph b/w torque & RPM

• **Table no.16 Viscosity of F6**

Spindle	RPM	Torque	Viscosity(Cp)
PD	12	65%	8125.4
PD	10	54.7%	5189.3
PD	8	45.6%	3438.8
PD	6	37.2%	1509.7
PD	4	11.1%	14.21
PD	2	3.7%	12.8

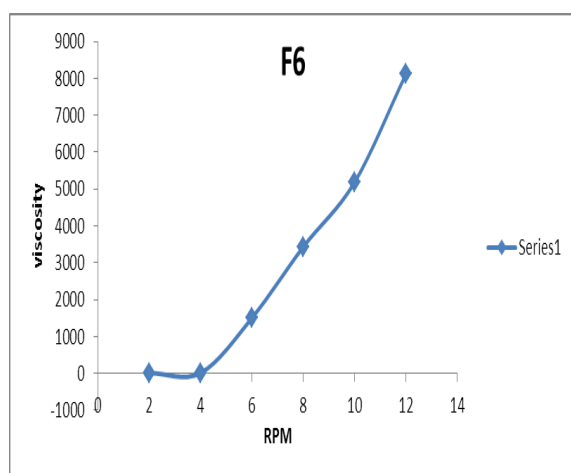


Figure 29 Graph b/w viscosity & RPM

Viscosity of all the six formulation was measured in Fungilab viscometer at different RPM (12, 10, 8, 6, 4 and 2) in table no.5.12-5.16 Firstly the effect of RPM on Escitalopram in-situ gel was observed it shows that as the RPM increase the viscosity also increased. Secondly the torque was measured against the rpm. As the rpm decreased the torque value is also decreased so, gel is converts from Newtonian to non- Newtonian behavior.

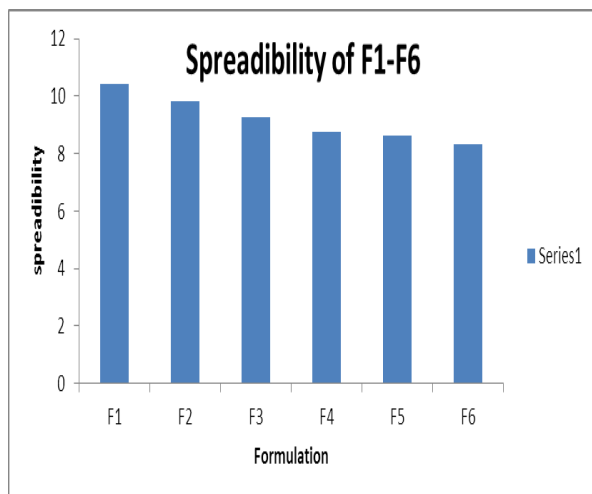
It is determined that rate of shearing is directly propositional shearing stress which shows that non-Newtonian in nature. When viscosity flow is increase the shearing rate also increases, hence it proves that formulation follows Dilatants flow and this originate the graph b/w RPM & torque.

Determination of spreadability

Spreadability of Escitalopram oxalate in-situ nasal gel is observed that as concentration of Carbopol-934 is increased spreadability decrease in table no.5.18. Hence value indicates that good spreadability which is desired for applicable of in-situ gel.

Table no.17 Spreadability for F1- F6

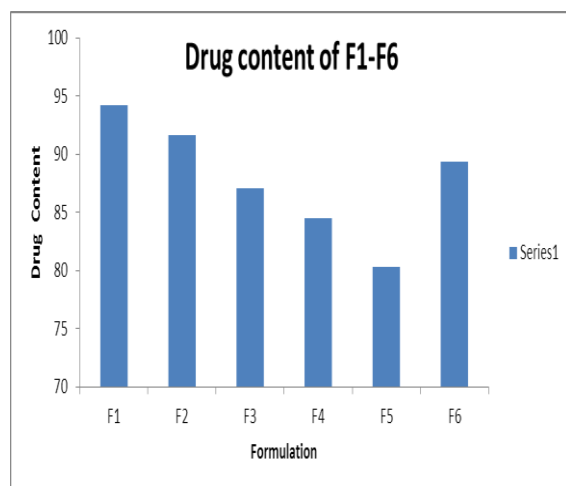
S.No.	Formulation	Spreadability
1.	F1	10.416±0.288
2.	F2	9.8039±0.133
3.	F3	9.2592±0.71
4.	F4	8.771±0.63
5.	F5	8.6206±0.42
6.	F6	8.3314±0.54

**Figure 32 Spreadability bar diagram****Measurement of Drug content**

Drug content of prepared nasal Insitu gel of all the six formulation was found to be have higher range between 94.17- 80.25% shown in table no.14 which is observed under UV spectroscopy.

Table no.14 Drug Content

S.No	Formulation	Drug content
1	F1	94.17±0.155
2	F2	91.60±0.235
3	F3	87.06±0.047
4	F4	84.46±0.27
5	F5	80.25±0.19
6	F6	89.37±0.142

**Figure no.34 Drug content bar diagram****Determination of in-vitro diffusion study**

In-vitro diffusion study was carried out in Franz diffusion cell using different dialysis membrane in different concentration it was observed that higher molecular weight of dialysis membrane slower release of drug. The observed data clearly shows that release rate of drug were related to polymer concentration shown in table 5.20 as the concentration of polymer increases so release rate of drug decreases. From the release data of the different formulation it was observed that released rate and cumulative percentage drug release were directly related to the nature of polymer and its amount.

- It is found that formulation F1 which is containing Carbopol-934P (0.1%) shows 94.05% drug releases (shows the highest release) in 4hrs. Formulation is best.
- Formulation F3 which containing Carbopol-934P (0.3%) show 89.18% drug release (show the good release) in 4 hrs.
- Formulation F6 which is containing Carbopol-934P (0.6%) show 77.72% drug release (show the moderate release) in 4 hrs.

As a result formulation of F1-F6 shows the concentration of Carbopol-934 various from 0.1 to 0.6 it was clearly observed that as the polymeric concentration increases the release of Escitalopram was decreased significantly up to 4 hrs. Hence it is concluded that F1 formulation is best for drug release.

In the Kinetic studies of all the formulation 'r' value of Zero order kinetic were the ranges from 0.950-0.991 to; similarly the 'r' First order kinetic were ranges from 0.965-0.976; Huguchi matrix 'r' range from 0.982 – 0.950; Korsmeyer peepes 'n' 0.988 – 0.950.

From the kinetic models it is observed that the Huguchi model is dominant for the release of Escitalopram oxalate from in-situ gel and the mechanism of release is non-fickian.

Table no.15 In-vitro Diffusion study

Time(min)	Cumulative Drug Release (%) ($\pm$ S.D)					
	F1	F2	F3	F4	F5	F6
15	16.23 $\pm$ 0.23	12.17 $\pm$ 0.19	9.37 $\pm$ 0.41	7.27 $\pm$ 0.3	5.32 $\pm$ 0.14	4.67 $\pm$ 0.70
30	22.04 $\pm$ 0.12	20.12 $\pm$ 0.3	15.48 $\pm$ 0.18	13.06 $\pm$ 0.02	10.27 $\pm$ 0.11	8.17 $\pm$ 0.04
45	30.14 $\pm$ 0.31	26.77 $\pm$ 0.17	29.79 $\pm$ 0.04	27.77 $\pm$ 0.17	23.13 $\pm$ 0.32	17.39 $\pm$ 0.51
60	42.01 $\pm$ 0.08	34.17 $\pm$ 0.26	36.27 $\pm$ 0.24	39.47 $\pm$ 0.27	36.17 $\pm$ 0.21	21.72 $\pm$ 0.09
90	51.27 $\pm$ 0.17	47.05 $\pm$ 0.27	49.21 $\pm$ 0.03	43.21 $\pm$ 0.4	47.01 $\pm$ 0.04	39.82 $\pm$ 0.1
120	68.14 $\pm$ 0.24	59.64 $\pm$ 0.38	54.02 $\pm$ 0.12	50.01 $\pm$ 0.12	51.13 $\pm$ 0.01	48.31 $\pm$ 0.01
150	75.27 $\pm$ 0.13	79.34 $\pm$ 0.24	74.21 $\pm$ 0.11	69.61 $\pm$ 0.01	65.25 $\pm$ 0.17	54.02 $\pm$ 0.12
180	81.6 $\pm$ 0.36	81.46 $\pm$ 0.32	79.11 $\pm$ 0.03	71.02 $\pm$ 0.04	68.21 $\pm$ 0.04	60.72 $\pm$ 0.54
210	85.7 $\pm$ 0.24	86.14 $\pm$ 0.14	84.27 $\pm$ 0.15	87.34 $\pm$ 0.75	75.02 $\pm$ 0.27	75.88 $\pm$ 0.92
240	94.05 $\pm$ 0.4	92.70 $\pm$ 0.06	90.80 $\pm$ 0.21	89.18 $\pm$ 0.43	80.72 $\pm$ 0.41	77.05 $\pm$ 0.08

Table no.16 T_{50} & T_{80} of In-vitro release

Formulations	T_{50}	T_{80}
F1	90,51.27	150,79.34
F2	90,47.05	150,79.34
F3	90,49.21	180,79.11
F4	90,43.21	180, 71.02
F5	120,51.13	180, 80.13
F6	120,48.31	180,75.88

Table no. 17 Kinetic model

Formulation code	r^2			Best fit model	Mechanism of model	Slope value(n) Korsmeyer peppas
	Zero order	1 st order	Huguchi matrix			
F1	0.950	0.965	0.982	Huguchi matrix	Non-fickian	0.804
F2	0.964	0.973	0.966	Huguchi matrix	Non-fickian	0.978
F3	0.966	0.924	0.988	Huguchi matrix	Non-fickian	0.811
F4	0.972	0.945	0.975	Huguchi matrix	Non-fickian	0.698
F5	0.980	0.990	0.984	1 st order	Non-fickian	0.757
F6	0.991	0.976	0.985	Zero order	Non-fickian	0.669

CONCLUSION

The aim of present work was used to prepare, evaluate and optimized Insitu nasal gel of Escitalopram oxalate for treatment of depression. Thermosensitive polymer (Poloxamer-188) and mucoadhesive polymer (Carbopol - 934P) were used for the preparation of effective Intranasal drug delivery system.

This study clearly demonstrated that the Escitalopram can be successfully delivered through nasal route by preparing the Insitu nasal gel. The gel was non-irritating and when it delivered through nasal mucosa provides an added advantage of bypassing the hepatic first pass metabolism. Through this method it is possible to control depression at faster rate of delivery as it able to bypass the Blood brain barrier. By formulating we can achieve higher bioavailability, allowing low dose, modified release pattern and reducing dosing frequency etc.

The main advantages of intranasal delivery are considerable. It is both rapid and non-invasive. It bypasses the Blood brain barrier and targets the central nervous system, reducing systemic exposure and thus systemic side effects. Even for drugs that can cross the

Blood brain barrier, it can reduce systemic side effects by reducing the need for the drug to enter the systemic circulation. It does not require any modification of the therapeutic agent being delivered and should work for a wide range of drugs. Intranasal delivery may facilitate the treatment and prevention of many different neurologic and psychiatric disorders.

In the present study in order to reach the goal of achieving the sustain release, thermoreversible polymers pluronic F 68(Poloxamer188) and were used along with mucoadhesive polymer Carbopol 934P. In addition good results were obtained with pluronic F 68 (20%) & Carbopol 934 (0.1%).

For optimizing drug and polymer ratio of six formulation F1-F6 were prepared varying the concentration of each polymer, they showed desirable pH, gelation forming temperature, gel melting temperature, drug content, spreadability and in vitro diffusion study and many other tests were performed to get the desired formulation which achieves for the intranasal delivery.

In vitro drug releases of the floating in situ gel were carried in phosphate buffer 6.4 from 0 to 4 hrs. Study was done in Franz Diffusion membrane apparatus to compare the release rate of formulation Prepared by using different ratio of polymers. In vitro drug release was conducted shown in 15.

- It is found that formulation F1 which is containing Carbopol-934P (0.1%) shows 94.05% drug releases (shows the highest release) in 4hrs. Formulation is best.
- Formulation F3 which containing Carbopol-934P (0.3%) show 89.18% drug release (show the good release) in 4 hrs.
- Formulation F6 which is containing Carbopol-934P (0.6%) show 77.72% drug release (show the moderate release) in 4 hrs.

Hence conclusion is all formulation data lies in the range from 94.5 – 77.05% and F1 formulation was 94.5, 92.70, 90.80, 89.18, 80.72 and 77.05% so, F1 is best formulation for drug release.

REFERENCE

1. Dey S. Mahanti B., Mazumder B., Malgope A., Dasgupta S. "Nasal drug delivery: An approach of drug delivery through nasal route", *Der pharmacia sinica*, 2011; 2(3): 94-106.
2. M. Alagusundaram, B. Chengaiah, D. Dhachinamoorthi. *International Journal of Research and Pharmaceutical Sciences*, 2010; 1(4): 454-465.
3. C.R. Behl, H.K. Pimplaskar, A.P. Sileno, J. de Meireles, V.D. Romeo. *Advanced Drug Delivery Reviews*, 1998; 29: 89-116.
4. Miyazaki S., Hirotsu A., Kawasaki N., Wataru K. and Attwood D. In situ gelling gellan formulations as vehicles for oral drug delivery. *J. Control Rel.*, 1999; 60: 287-295.
5. Michael I. Ugwoke, Remigius U. Agu, Norbert Verbeke, Renaat Kinget. *Advanced Drug Delivery Reviews*, 2005; 57: 1640-1665.]
6. Lisbeth Illum. *Drug Discovery Today*, 2002; 7(23): 1184-1189.
7. Kamble M.S., Bhalerao K.K., Bhosale A.V., Chaudhari P.D., "A Review on Nose-To-Brain Drug Delivery," *International Journal of Pharmaceutical and Chemical Sciences*, 2013; 2(1): 516-525.
8. Chavan P. Dhole S., Yadav M., "nasal drug delivery system: a review" *world journal of pharmacy and pharmaceutical sciences*, 2014; 3(12): 598-617.
9. Ferry, Jhon D. *Properties of polymers*. New york; wiley, 1980.
10. http://www.corporate.basf.com/basfcorp/img/stories/wipo/haargel_e.pdf.
11. Van Esch J, Schoonbeek F, De Loos M, Veen EM, Kellog RM, Feringa BL. Low molecular weight gelators for organic solvents. In: ungaro R, Dalcanele E, editors. *Supramolecular science. Where it is and where it is going*. Kluwer Academic Publishers, 1999; 233-259.
12. Kumar R, Katore OP, Lecithin organogels as a potential phospholipid-structured system for topical drug delivery: A review. *American association of pharmaceutical scientists Pharm Sci Tech*, 2005; 6: E298-E310.
13. Carreti E, Dei L, Weiss RG. Soft matter and art. *Conversation. Rheoreversible gels and beyond. Soft Matter*, 2005; 1: 17-22.
14. Perneti M, van Malssen KF, Floter E, Bot A. structuring of edible oil by alternatives of crystalline fats. *Current opinion in colloid and interface science*, 2007; 12: 221-231.
15. Chavan P. Dhole S., Yadav M., "nasal drug delivery system: a review" *world journal of pharmacy and pharmaceutical sciences*, 2014; 3(12): 598-617.
16. Mehta MR, Surve SA et al. *Ind J Pharm Sci.*, 2010; 59: 153-180.
17. Bajpai V. "In situ Gel Nasal Drug Delivery System – A Review", *International Journal of Pharma Sciences*, 2014; 4: 577-580.
18. Aishwarya J Jadhav, Sheetal B Gondkar, Ravindra B Saudagar. A Review on nasal drug delivery system. *World journal of pharmacy and pharmaceutical sciences*, (WJPPS), 2014; 3(8): 231-254.
19. Anwar A. Hussain *Intranasal drug delivery Advanced Drug Delivery Reviews*, 1998; (29): 39-49.
20. Dey S. Mahanti B., Mazumder B., Malgope A., Dasgupta S. "Nasal drug delivery: An approach of drug delivery through nasal route", *Der pharmacia sinica*, 2011; 2(3): 94-106.
21. Niels Mygind and Ronald Dahl, *Anatomy, physiology and function of the nasal cavities in health and disease Advanced Drug Delivery Reviews*, 1998; (29): 3-12.
22. Kamble M.S., Bhalerao K.K., Bhosale A.V., Chaudhari P.D., "A Review on Nose-To-Brain Drug Delivery," *International Journal of Pharmaceutical and Chemical Sciences*, 2013; 2(1): 516-525.
23. Adams, G.L., Boies, L.R. and Hilger, P.A., 1989. Boies's *Fundamentals of Otolaryngology a Textbook of Ear, Nose and Throat Diseases*, Saunders, Philadelphia, 177-195.
24. Ramya D. D., Abhirami M., Brindha R., Gomathi S., Vedhahari B.N., "In-Situ Gelling System – Potential Tool For Improving Therapeutic Effects Of Drugs", *International Journal Of Pharmacy And Pharmaceutical Sciences*, 2013; 5(3): 27-30.
25. Pires A., Fortuna A., Gilberto A., Amilcar F., "Intranasal Drug Delivery: How, Why and What for?" *Journal Pharmaceutical Science*, 2009; 12(3): 288-311.
26. Kaliner, M., Marom, Z., Patow, C. and Shelhamer, J., *Human respiratory mucus. J Allergy Clin Immunol*, 1984; 73: 318-323.
27. Swamy N G N, Zaheer A, "Mucoadhesive In situ Gels as Nasal Drug Delivery Systems: An Overview", *Asian Journal of Pharmaceutical Sciences*, 2012; 7(3): 168-180.

28. Graff, C. L. and Pollack, G. M., Nasal drug administration: Potential for targeted central nervous system delivery, *J Pharm Sci*, 2005; 94: 1187-1195.
29. Pardridge, W. M., Blood-brain barrier delivery, *Drug Discov Today*, 2007; 12: 54-61.
30. Mistry, A., Stolnika, S. and Illum, L., Nanoparticles for direct nose-to-brain delivery of drugs, *Int J Pharm*, 2009; 379(1): 146-157.
31. Sharma, R., Nasal Mucosa a novelistic platform for brain targeting, *American Journal of Pharm Tech Research*, 2012; 2(1): 2249-3387.