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Formulation and Characterization of Tranylcypromine loaded Polymeric Micellar In-Situ Nasal Gel for treatment of Depression

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Abstract:*Tranylcypromine is a drug used as antidepressant,anxiolytic, nonselective MAO A/B inhibitor. This drug is used to treat depression.The research was conducted to develop a polymeric micelle using a block copolymer, Pluronic F-68 and Gelucire 50/13 to improve the permeability of Tranylcypromine (TCP). A direct dissolution method was used to prepare polymeric micelles. The prepared micelles were characterised for particle size, % EE, zeta potential, in-vitro release. These micelles solution was used to prepare in situ gel by cold method in order to achieve controlled release. Central composite design was used for optimization of both polymeric micelles and in-situ nasal gel.The main objective of this research work is to develop formulation acting centrally without undergoing first pass metabolism i.ie. directly nasal to brain delivery route.*

Keywords*: Tranylcypromine, Polymeric micelles , Direct dissolution method, In situ nasal gel, cold method*

I. Introduction

Now a days more than 254 million people are suffered from depression. Depression may sometimes leads to suicide. In order to treat depression brain targeted drug delivery system is used. The Brain Targeted Drug Delivery System in which delivery of drugs to the CNS is used for treating specific neurological disorders and various diseases such as Meningitis, Bipolar depression, Mania, Encephalitis, degenerative diseases such as Alzheimer, Parkinson and tumours such as Glioblastoma.[1]According to WHO, Depression is a common mental disorder that characterized with-a depressed mood, loss of interest or pleasure, feelings guilty or low self- worth, disturbed sleep or appetite, low energy, and poor concentration.[2]Depression is characterized by decreased in mood or loss of interest which mainly occurs due to decrease in the mood elevating neurotransmitters i.e. (Biogenic amines) like non-adrenaline(NA) and serotonin (5-HT) in the region of hippocampus region. It usually results in an adverse life events, such as losses of a significant person, object, relationship or health, but it can also occur due to no apparent cause.[3] These problems can become chronic or recurrent and lead to substantial impairments in an individual's ability to take care of his or her every day responsibilities.[4]. In order to treat depression we have to increase the levels of NA and 5-HT. Tranylcypromine – an antidepressant non-reversible MAO inhibitor is used to treat depression in the form of polymeric micelles. It is a quickest acting potent drug. Tranylcypromine irreversibly and nonselectively inhibits MAO within neurons. MAO appears to regulate the levels of monoamines released on synapticfirings.By using the polymeric micellar in-situ nasal gel formulation, an attempt was made to increase its permeation so as to increase its bioavailability by avoiding first pass metabolism.

Depression is mainlyoccurs due to low levels of monoamines, the inhibition of MAO serves to ease the depressive symptoms, as this results in the increase in the concentration of these amines within CNS.

Polymeric micelles are the self-assembly of amphiphilic block copolymers into an aqueous solution. Polymeric micelles are nano sized assemblies of amphiphilicblock copolymers exhibiting an unique core corona structure.[figure 1] The hydrophilic part is very important to stabilize the micelles in aqueous environment. It also minimizes clearance by the mononuclear phagocytic system (MPS) after systemic administration whereas the hydrophobic core function as a drug[5].By using the polymeric micelles solution in situ nasal gel is prepared. In Situ

forming drug delivery systems is the system in which drug is in solution from before administration in the body, but once administered, it converts into gel.[6] This study is designed with the aim to enhance the bioavailability of BCS class III drug.This study mainly focuses on the use of the non-ionic surfactant and block polymer as a penetration enhancer to increase the permeability across BBB.

II. Materials and method

Materials

Drugs: Tranylcypromine- Obtained as a gift sample from Glenmark Industries pvt.Ltd., Mumbai

Chemicals: Gelucire50/13, Pluronic F-68, Pluronic F-127, HPMC K-100, PEG-400, Tween 20, Triethanolamine, benzalkonium chloride, are purchased from sigma Aldrich.

Solvents: Phosphate buffer of pH 6.5(All other chemicals for buffer solution were analytical grade.)

Preformulation study:

Preformulation study is the primary stage in the objective development of the dosage form of drug. It's used to characterize the drug for its physical and the chemical properties and also used to determine the compatibility of the drug and the excipient.

Study of Block Polymers for Solubilization (CMC)

Surface tension method was used for CMC determination of Pluronic F68 and Gelucire 50/13 polymers. The CMC of block copolymer at 25°C was determined in pure water. The CMC determination for Pluronic F68, Gelucire 50/13 were based on the change in surface tension with surfactant concentration.Stalagmometer was used to measure the surface tension. Each surface tension measurement was repeated three times, and the typical error in the CMC determination was less than 5%.

Cloud point (CP)

Cloud point of optimized formulation with or without drug were determined in the presence and absence of varying amount of added sodium chloride by gradually heating the solution in thin 20 mL glass tubes. Glass tubes were immersed in a temperature-controlled water bath. The solutions were stirred with amagnetic stirrer bar during heating. The heating rate of samples were adjusted to 1°C/min. The first appearance of turbidity was taken as cloud point and cloud point values were found to be reproducible within 0.5°C.

Methods

Two methods are used. One for preparation of polymeric micelles and one for preparation of in situ nasal gel ,i.e. Direct dissolution method and Cold method resp.

a. Direct dissolution method [7]

Polymeric micelles containing Tranylcypromine were prepared by direct dissolution technique using block copolymer (Pluronic F 68 and Gelucire 50/13) alone. Block copolymers was dissolved in water, Tranylcypromine was then added to the copolymer solution. The solution was stirred continuously on a mechanical stirrer at room temperature for different time interval at different RPM for preparation of micelles. Nonincorporated drug was separated by filtration of micelle suspension through a 0.2µm filter. The filtered solution was diluted 10 times with polymer solution. The Tranylcypromine concentration in filtered solution was estimated by measuring UV absorbance at λmax 264nm.

b. Cold method [8]

The **cold method** has been chosen for the preparation of gels.First of all,16 % of Pluronic was used for the preparation of gels because at this concentration sol converted into gel at nasal temperature. The required amount of HPMC K-100 dissolved in a 10 ml polymeric micelles solution by using magnetic stirrer. After that add required quantity of PEG-400 and benzalkonium chloride was added.pH of resultant solution was adjusted to 6.5 using triethanolamine. Prepared mixture was kept at 5°C overnight to obtain clear solution.

Characterizationof polymeric micelle

1. Solubility study

The solubility of Tranylcypromine was determined in various polymers and non-ionic surfactant Poloxamer: water (1:5 weight ratio) and by dissolving excess amount of a Tranylcypromine in 5 ml of surfactant mixture using a stirrer on horizontal shaker for 24 hours at room temperature. The equilibrated samples were then centrifuged at 6000 rpm for 30 min. to remove undissolved drug, then the clear supernatant liquid was decanted. The solubility of Tranylcypromine was measured by validated UV spectrophotometric method at 264 nm.[9]

2. Fourier Transform Infrared Spectroscopy (FTIR):

The compatibility of Tranylcypromine with different excipient used in the formulations was explored using FTIR. The FTIR spectra of plain and physical mixtures of TCP and copolymers (at 1:1w/w ratio) were recorded on FTIR spectrophotometer (Perkin-Elmer 4000 USA) in the range 0f 4000-400 cm^{-1}.[10]

3. Differential Scanning Calorimetry (DSC) Analysis:

Thermal properties of plain TCP and different excipient used in the formulations were investigated using Differential scanning calorimetry (Lab- METTLER) . Accurately weighed samples were placed in hermetically closed aluminum pans, and the empty aluminum pan were used as a reference. The samples were heated at a heating rate 10°C per min. upto 300°C under inert nitrogen atmosphere.[11]

4. **Determination of percent entrapment:**

The filtered solution of a drug containing polymeric micelles solution was diluted 10 times with a distilled water. The Tranylcypromine concentration in polymeric micelles solution was estimated by measuring UV absorbance at λmax 264 nm. After the drug contents by UV, incorporated drug(%) and drug weight (%) in micelles were calculated by using equation (1) and (2):

Drug incorporated (%) =(a/b)*100……(1)

Drug weight in micelle =(a/b+c)*100……(2)

Where,

a – amount of drug loaded in micelle(mg)

b – amount of drug used in micelle preparation (mg)

c – amount of polymer used in micelle preparation (mg).[12]

5. Determination of particle size:

The micelle size determination was performed by using Digital microscope. 1 ml of micelle solution was diluted with water for 10 times slowly to form dispersion. Diluted sample directly placed on a slide and placed below the microscopic lens for measurement. All measurements were performed in triplicates.[13]

6. In vitro Release Study:

The release of Tranylcypromine from polymeric micelles was determined using themembrane diffusion technique. 1 ml of polymeric solution was placed in a diffusion cell of diameter 2.5 cm, and having volume 15 ml . The lower open end of glass tube was covered with soaked cellulose membrane. The cell then filled with phosphate buffer solution of pH 6.5. Then the cell was constantly stirred at speed of 250 rpm at 37 ± 10^{0C}on a magnetic stirrer. Samples were withdrawn at 15, 30, 45, 60, 90, 120, 150, 180,210,240 min (upto 8 hours) intervals and replaced simultaneously with a equal volume of fresh phosphate buffer solution. The Tranylcypromine concentration in the samples was analysed spectrophotometrically, at wavelength 264 nm. Release profile were expressed regarding % cumulative release *vs* time [14]

7. Stability study of micelles

The micellar formulations were stored at 5±3°C for one month. After 15 to 30 days the formulations were analysed at % EE using UV spectrophotometry as an analytical method. 1 ml of formulations was centrifuged, and supernatant was diluted upto 10 ml with distilled water, sonicated for 10 min. and analysed for TCP remaining.[15]

Characterization of In-situ Gel.[16]

1. Clarity

The formulations were inspected visually for clarity , colour in sol and gel form against white background any for any particulate matter if present.

2. pH of gel

pH of formulations was measured using pH meter which was previously calibrated by using standard buffers of pH 7.

3. Spreadability

Spradability was determined by using a 10^x4 rectangular glass slide. 1 drop of gel was placed on the slide and it was covered by another slide having same dimension. Spreadability was determined by relative distance travelled by drop of gel before its gelation. Average of three readings was recorded.

4. Drug content

1 ml of formulation of gel was taken in 10 ml volumetric flask. Then it was diluted to10 ml with distilled water then volume adjusted to 10 ml. From this 1 ml solution was taken and again diluted with distilled water up to 10 ml. After this absorbance of prepared solution was measured at particular wavelength of the drug by using UV Visible spectrophotometer.

5. Viscosity

The rheological studies were carried out using the Brookfield viscometer. The gel formulation under study was placed in sample holder and then spindle #4 was selected and inserted perpendicular into the sample.

6. In vitro diffusion study of in situ gel

10 mg of gel was placed in donor chamber, of Franz having capacity 2.4 diameter and 15 ml diffusion cells. Cellophane membrane with diffusion area 2 cm^2 used. Phosphate buffer of pH 6.5 was prepared in which membrane soaked. Phosphate buffer was placed in acceptor membrane. Temperature is maintained at 37°C±0.5°C. 1 ml sample was withdrawn from acceptor chamber and replaced the sample volume with equal amount of phosphate buffer after each sampling process. Study was carried out for 8 hrs.

7. In-Vitro Permeability Study

It is calculated by using formula:

Drug permeability={X}*(1/(Z)*{Y}*60)

Where,

X=Steady state appearance, i.e. Slope

Y=Area exposed to the tissue

z=Drug conc.in donor compartment

III. Result and Discussion

Optimization of polymeric micelles:

Thirty-five Tranylcypromine polymeric micelles formulation were prepared according to factorial design employing the factors and levels shown in (Table 1). And thirteen formulation of further converted in-situ nasal gel are prepared (Table 2) according to the design expert software12.

Factors and Levels for 35 formulations			
Independent variables	**Parameter**	**Level**	
		-1	**+1**
A	Conc. Of block polymer	1	3
B	Conc. Of surfactant	1	3
C	Effect of RPM	200	1000
D	Time of stirring	6	28
E	Effect of sonication	With	Without
F	pH	5.5	6.5
G	Type of block polymer	Gelucire 50/13	PluronicF-68
Dependant variables			
Response 1	% Drug loading		
Response 2	% Drug Release		

Table 1. Dependent and independent variables for polymeric micelles

Experimental design for polymeric micelles according to Central Composite Design.							
Batch code	**Variables level in coded form**						
F	**A**	**B**	**C**	**D**	**E**	**F**	**G**
F1	1	1.96	600	28	With	6.5	Gelucire50/13
F2	1	1	852	27.56	With	5.5	Pluronic F-68
F3	1	3	772	6	Without	6.5	Gelucire50/13
F4	3	3	1000	27.56	With	5.5	Pluronic F-68
F5	2.35	1	848	11.5	With	5.5	Gelucire50/13
F6	1.67	2.01	1000	15.68	With	5.5	Pluronic F-68
F7	3	1.75	600	6	With	6.5	Gelucire50/13
F8	3	3	1000	28	With	6.5	Gelucire50/13
F9	1	1.05	1000	6	With	6.5	Gelucire50/13
F10	1.97	1.90	600	21.18	With	5.5	Pluronic F-68
F11	3	3	600	28	With	5.5	Gelucire50/13
F12	2.9	3	1000	28	Without	5.5	Gelucire50/13
F13	1	3	1000	28	With	5.5	Gelucire50/13
F14	3	1	600	28	With	6.5	Pluronic F-68
F15	1	1.99	600	6	With	5.5	Gelucire50/13
F16	1	1	600	6.55	Without	6.5	Pluronic F-68
F 17	3	1	1000	6.55	With	6.5	Pluronic F-68
F18	1.9	1.85	766	6	Without	5.5	Pluronic F-68
F19	1.73	1	600	28	Without	5.5	Gelucire50/13
F20	3	3	750	6.44	Without	6.5	Pluronic F-68
F21	1.87	3	774.33	15.34	With	5.5	Gelucire50/13
F22	1	1	1000	28	Without	6.5	Gelucire50/13
F23	1	1	1000	6	Without	5.5	Gelucire50/13
F24	3	2.4	600	28	Without	6.5	Gelucire50/13
F25	1	3	680	7.43	With	6.5	Pluronic F-68
F26	1	3	680	7.43	With	6.5	Pluronic F-68
F27	3	2.45	600	6	Without	5.5	Gelucire50/13
F28	3	1	1000	27.89	With	5.5	Gelucire50/13
F29	3	1	600	6	With	5.5	Pluronic F-68
F30	3	1	1000	6	Without	6.5	Gelucire50/13
F31	3	3	1000	6	With	5.5	Gelucire50/13
F32	3	1	922	26.46	Without	5.5	Pluronic F-68
F33	1	3	1000	6	Without	5.5	Pluronic F-68
F34	1	3	1000	28	Without	6.5	Pluronic F-68
F35	1	3	600	27.45	Without	5.5	Pluronic F-68

Table 2. TheCentral Composite design for polymeric micelles in terms of coded factors.

Table 3: Factors and Levels for 13 formulations			
Independent variables	**Parameter**	**Level**	
		-1	**+1**
A	PEG -400Conc.	4	8
B	HPMC K-100 Conc.	0.1	0.5
Dependant variables			
Response 1	Spreadability		
Response 2	Permeation		
Response 3	Viscosity		

Table 3. Dependent and independent variables for In-situ gel.

Experimental design for in-situ nasal gel according to Central Composite Design.		
Batch code	**Variables level in coded form**	
F	A	B
F1	4	0.5
F2	6	0.0171573
F3	8.82843	0.3
F4	8	01
F5	6	0.3
F6	3.171576	0.3
F7	6	0.582843
F8	6	0.3
F9	6	0.3
F10	8	0.5
F11	4	0.1
F12	6	0.3
F13	6	0.3

Table 4. TheCentral Composite design for in situ nasal gel in terms of coded factors

Organoleptic properties of drug

The drug was characterized for the colour, odour, taste appearance and the result was recorded using the descriptive terminology, and the result is shown in Table no.3below:

Sr.No.	Parameter	Observation
1	Colour	White
3	Odour	Odourless
4	Appreance	Amorphous powder

Table 5: Organoleptic properties of drug

Solubility study

Solvent	Solubility(mg/mL)
Water	40
Methanol	4
Ethanol	Very slightly soluble
Diethyl Ether	0.5
Chloroform	Practically insoluble

Table 6: Solubility study of Tranylcypromine

Polymer	Pluronic F-68	7.05
	Gelucire 50/13	5.07
Surfactant	Tween 20	4.65

Table 7: Solubility study of polymer and surfactant.

Melting point of Drug

Melting point of the drug lopinavir was found to be 225ºC by capillary method.[19]

Fourier transform Infrared spectroscopy

The IR of drug was found to be similar to the standard IR Spectrum of Tranylcypromine which indicates that the obtained drug sample was pure. (Fig-3)

The FTIR spectra of pure drug Tranylcypromine and physical mixture of Tranylcypromine samples are shown (Fig.4). The characteristics of IR absorbance peak of Tranylcypromine compared with IR spectra of Tranylcypromine physical mixture, which shows similarity in peaks of physical mixture and indicated no interaction between drug and polymers.

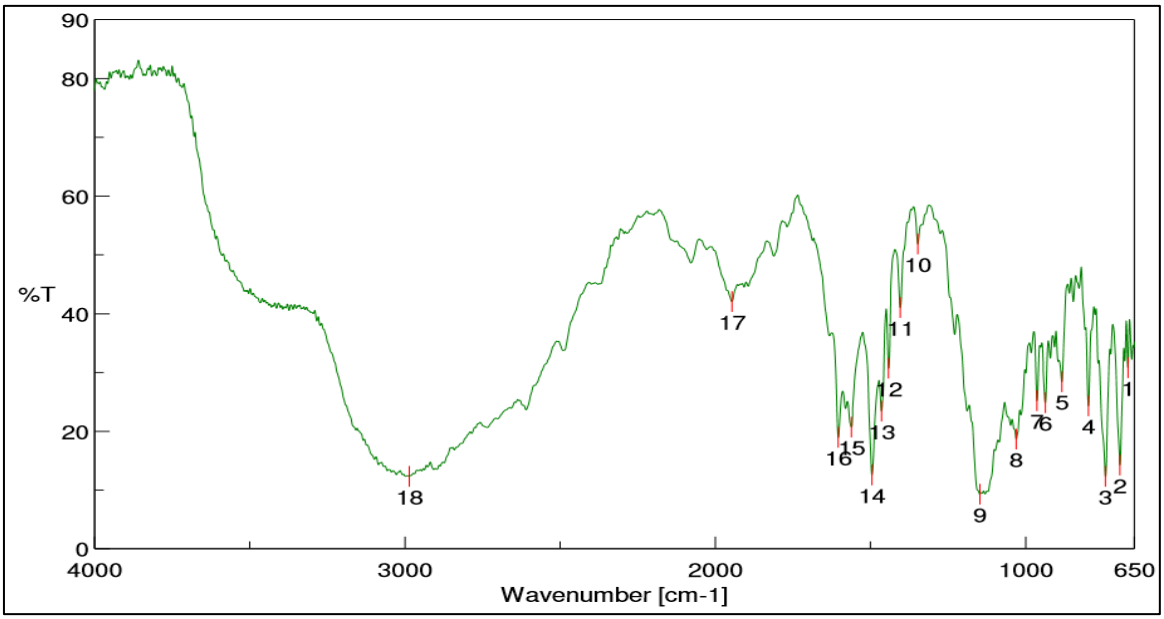

Fig.3:IR spectra of Tranylcypromine

IR Signals(cm^{-1})	Observed Signals (cm^{-1})	Remarks
675-1000	883	=C-H bending
1350-1480	1403	-C-H bending
1400-1600	1464	C=C stretching
1550-1640	1562	N-H bending
2950-3000	2986	C-H stretching

Table 8.IR spectra of Tranylcypromine with excipient mixture

DSC Study of drug Tranylcypromine

The purity of Tranylcypromine drug was confirmed by the Differential scanning calorimetry with the spectrum of the standard drug. The figure shows sharp endset at 225°C, corresponding to the melting temperature of drug, sharp peak of the drug shows purified form of the drug.And due to amorphous nature it undergoes degradation at higher temperature gives exothermic peak.

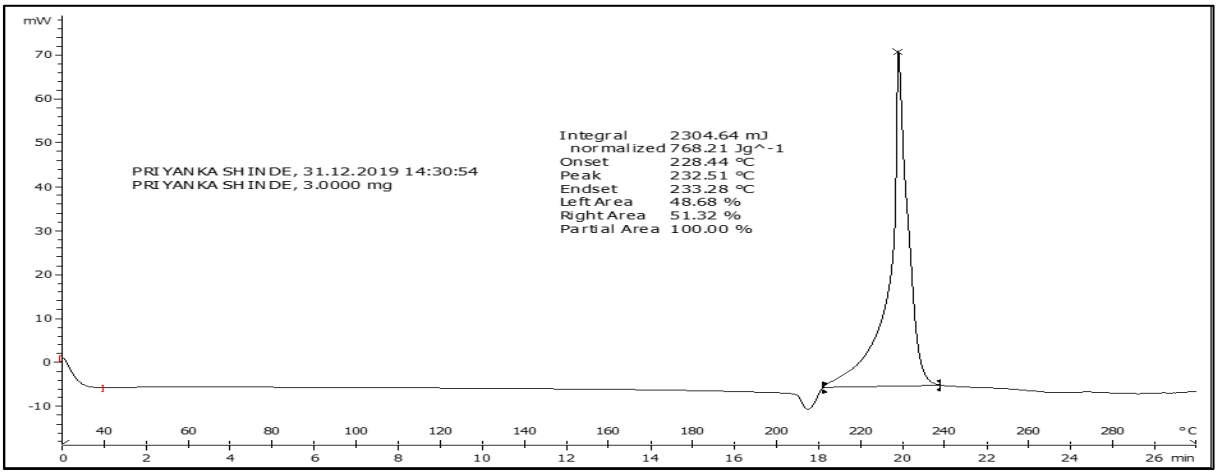

Fig.5: DSC of Tranylcypromine

Polymeric micelles

1. Effect on % Drug loading:

ANOVA test for observed data of % Drug loading indicates that the quadratic model was significant and fitting for the data. The model was found to be significant.

Response	**P value**	**F value**	**Equation**	**Significance**
%Drug loading	< 0.0001	45605.41	% Drug loading= -90.97+4.47A+6.95B-2.95C-1.48D-9.67E+7.47F+10.56G+14.21AB+4.30AC-1.83AD-2.15AE+4.00AF+4.30AG-44.28BC-14.03BD+2.01BF+8.53BF-	significant

			13.38BG+19.06CD+4.52CE+15.85CF+1.62CG -5.80DE+12.89DF+5.20DG+7.79EF+7.46EG- 5.37FG-97.30A^2+82.38B^2+85.45C^2+75.65D^2	

The % drug loading of the all the polymeric micelle batches were found between ranges of -2.01 ± 102.13%. From the result obtained from the Response surface plot was as found that the % drug loading increases in micelle using Pluronic f-68 as compared to Gelucire 50/13.

In polymeric formulation, addition of energy to obtain the micelles is provided by sonication. It observed with sonication it decreased in the vesicle size leads to increase in drug loading [100] As concentration of block polymer , time of stirring, and RPM increases , drug loading in the polymeric micelles solution increases . In the presence of surfactant Tween 20, percent entrapment increases while with presence of Gelucire 50/13 it decreases. Gelucire 50/13 is an excipient composed of fatty acid (C16 and
C18), PEG esters, and free PEG , whereas Tween 20 molecule has 20 repeated units of polyethylene glycol. So higher the carbon chain length, it plays role in increase in percent entrapment.

Increase in the concentration of surfactant beyond the critical micelle concentration, reduces the effectiveness in reducing the surface tension with a consequent increase in vesicle size and reduces entrapment efficiency. The another reason might be due to the surfactant there is possibility of formation of aggregates. This results in decrease in the percent entrapment [17].

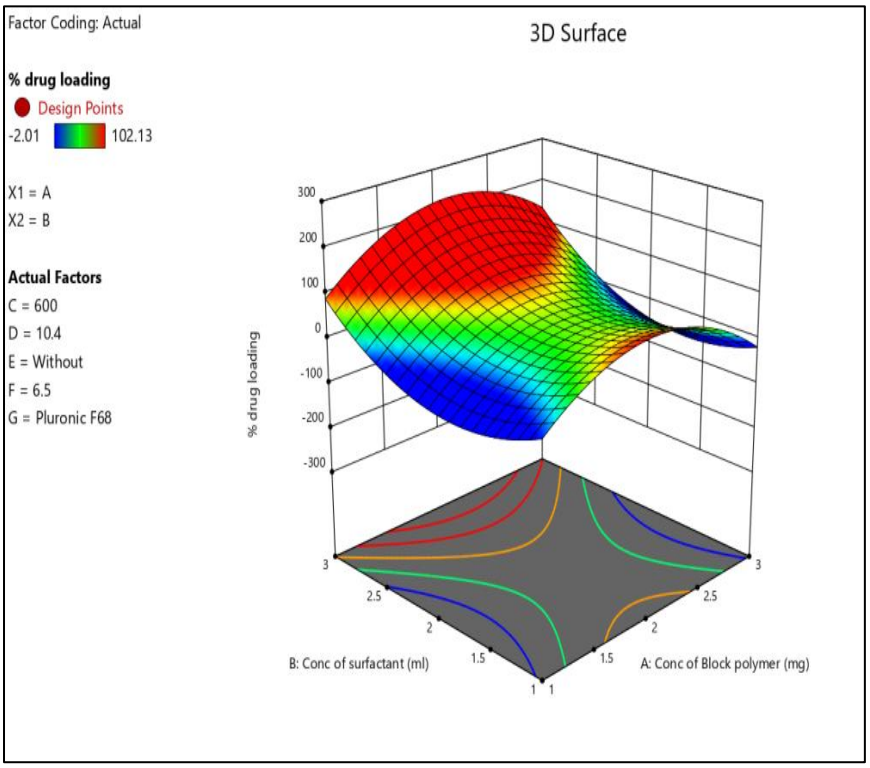

Fig.6: 3D Response Surface Plots for %Drug loading of Drug

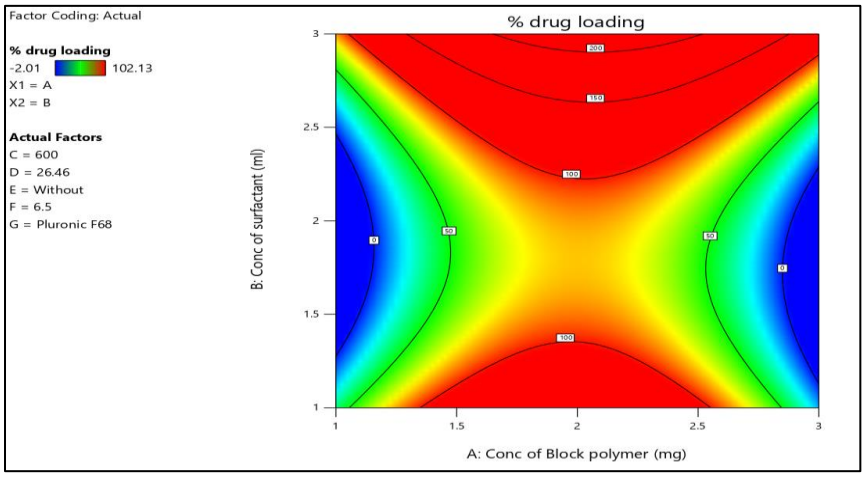

Fig.7:Counter Plots for %Drug loading of Drug

2. Effect on % Drug release:

ANOVA test for observed data of %Drug release indicates that the quadratic model was significant and fitting for the data. The model was found to be significant.

Response	P value	F value	Equation	Significance
%Drug release	< 0.0001	204.75	%Drug Release = +59.31-7.15A-6.55B+1.72C-0.3935D-1.50E+3.74F+3.11G+8.41AB+9.69AC-4.12AD+1.07AE+1.54AF-2.15AG-3.83BC+0.1033BD+1.98BE+0.3942BF-4.72BG-4.26CD+3.25CE-4.81CF+5.69CG+2.06DE+2.74DF-7.78DG+4.31EF-2.84FG-2.02FG.	significant

The % drug release of the all the polymeric micelle batches were found between ranges of 27.23 ± 99.65%. Drug release was more in the formulation containing Gelucire 50/13 as compared to the Pluronic F-68. As RPM of mechanical stirrer get increases drug release decreases slowly. It is due to the hydrophobicity or increase in the no.of carbon. The drug release is depend on amount of the drug and the molecular weight of the drug. The amount of drug present in micelles found to influence the release. The rate of diffusion of the drug micelles get influenced by the property of micelle core. The increase in the molecular weight of core forming block will increase the size of core provided hydrophilic block length constant. This increases the molecular weight of hydrophobic block and decreases the size core and thus decreases drug release [18]. The presence of electrolyte does not have effect on drug release, without sonication drug release increases and with sonication it decreases. With sonication the drug release decreases it may due to increasing interactions between micelle and core .

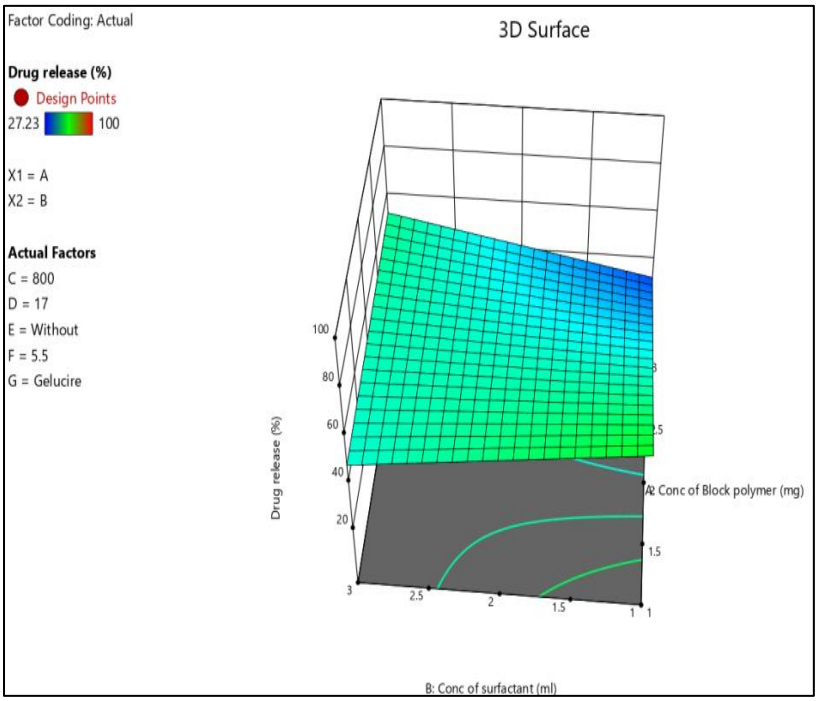

Fig.8:3D Response Surface Plots for % Drug release.

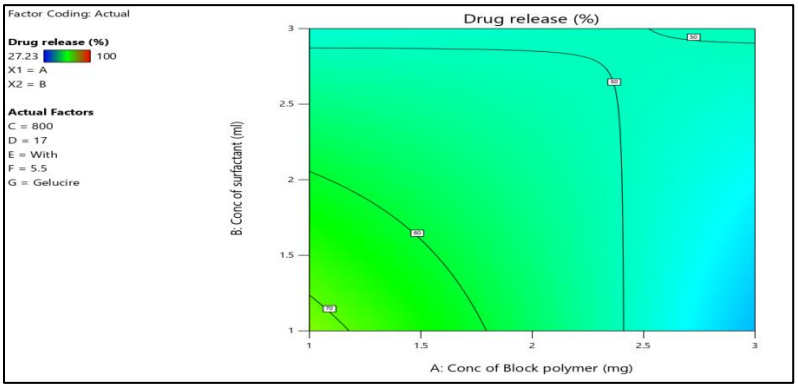

Fig.9: Counter Plots for %Drug release of Drug.

Permeability studies:

Formulation no.	Permeability[µg/cm^2/h]	Formulation no.	Permeability[µg/cm^2/h]
1	64.67±0.015	19	88.36±0.010
2	94.70±0.007	20	88.81±0.001
3	70.68±0.011	21	68.06±0.032
4	72.57±0.006	22	73.70±0.028
5	83.85±0.010	23	82.36±0.018
6	65.12±0.028	24	90.74±0.010
7	72.92±0.012	25	75.08±0.026

8	95.38±0.005	26	82.79±0.017
9	65.12±0.022	27	82.39±0.016
10	85.70±0.013	28	93.06±0.006
11	90.24±0.008	29	81.21±0.014
12	80.46±0.012	30	83.85±0.008
13	59.41±0.036	31	62.03±0.024
14	59.28±0.038	32	72.19±0.018
15	86.48±0.014	33	62.03±0.023
16	79.72±0.025	34	57.67±0.032
17	84.06±0.016	35	76.65±0.016
18	81.51±0.019		

Where,n=3, ±SD

Table 9. In vitro permeability calculations of polymeric micelles.

These are the calculations of all 35 batches from which formulation 8 is used for the further formulation of insitu nasal gel

Optimization of In-situ gel

Spreadability

1. **Effect on Spreadability:**
   ANOVA test for observed data of spreadability indicates that the quadratic model was significant and fitting for the data. The model was found to be significant.

Response	**P value**	**F value**	**Equation**	**Significance**
Spreadability	< 0.0001	533.99	**Spreadability**=+6.83-0.2931A+0.3463B-0.1250AB-0.5212A^2-0.4612B^2.	significant

The spread ability of formulation ranged in between 5.32-6.83 cm.

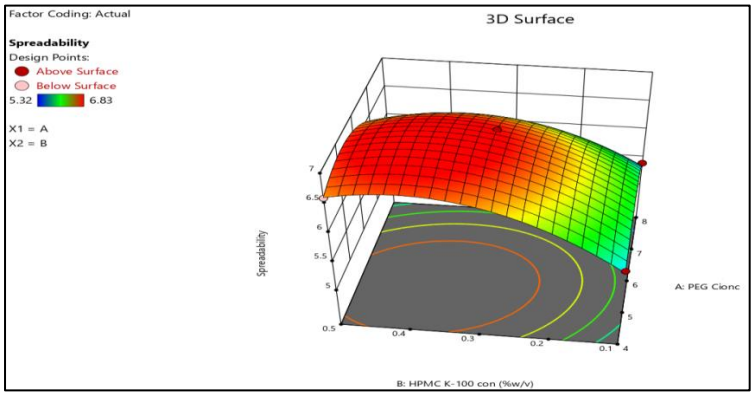

Fig.10:3D Response Surface Plots for Spreadability

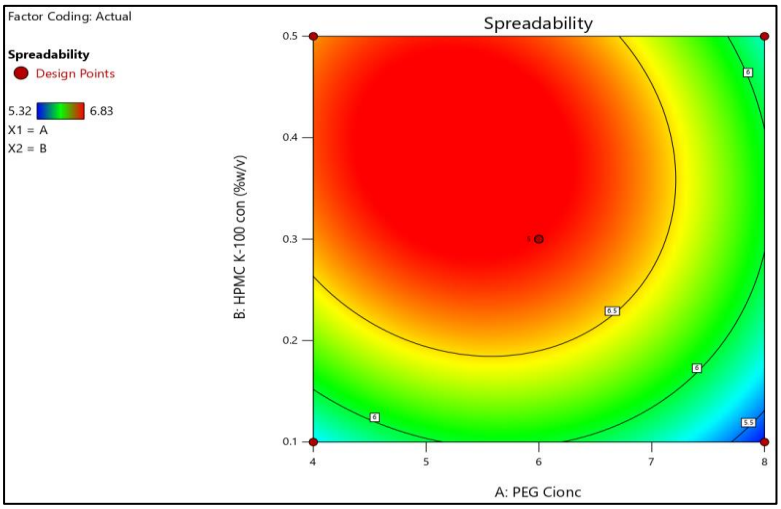

Fig.11: Counter Plots for Spreadability.

The spreadability of the in-situ nasal batches were found between ranges of 5.32 ± 6.83. It might be due to the as there is increase in the concentration of polymer, increase in the spreadability. but in case of HPMC K-100 it was found to be less spreadable due to the more viscous nature of HPMC K-100.

2.Permeation

Effect on Permeation

ANOVA test for observed data of permeation indicates that the quadratic model was significant and fitting for the data. The model was found to be significant.

Response	**P value**	**F value**	**Equation**	**Significance**
Permeation	< 0.0001	143.57	**Permeation=**+76.82-2.67A-6.71B-4.05AB+10.28A^2+0.0875B^2	significant

The Permeation of formulation ranged in between 69.32-102.02%

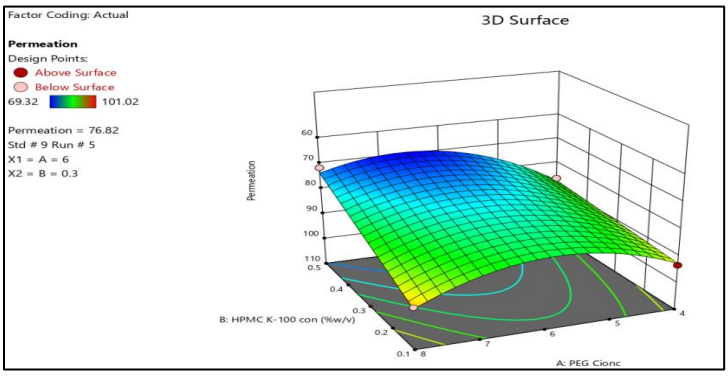

Fig.12:3D Response Surface Plots for Permeation.

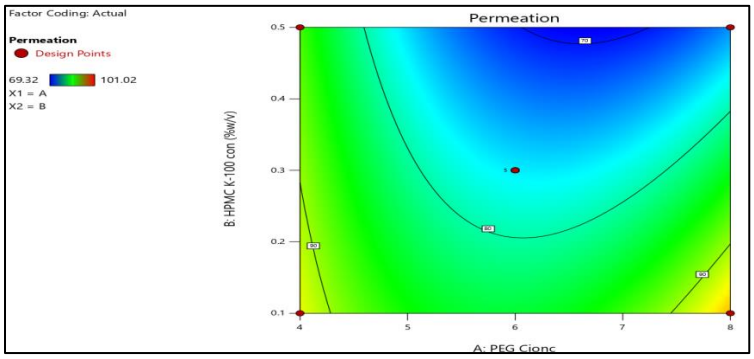

Fig.13: Counter Plots for permeation**.**

The permeation of the in-situ nasal batches were found between ranges of 69.32 ±101.02. But as we see in the above diagram increase in the concentration of HPMC K-100 there is decrease in the drug permeation. This might be due to the highly viscous nature of the HPMC K-100, it causes extensive swelling of polymer creates thick gel barrier for drug diffusion. As we increase the concentration of PEG -400, drug permeation also increases. This could be due to the dissolution of aqueous soluble fraction of the polymer matrix leads to decrease in mean diffusion path length of drug molecule to release in diffusion medium and hence cause higher release rate.

3.Viscosity

ANOVA test for observed data of viscosity indicates that the quadratic model was significant and fitting for the data. The model was found to be significant.

Response	P value	F value	Equation	Significance
Viscosity	< 0.0001	934.75	Viscosity=174.34A+23.62B+220.50AB-113.12A^{2}-198.12B^{2}.	significant

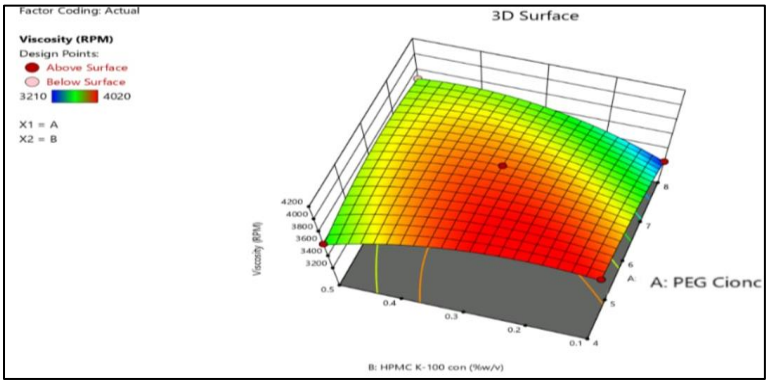

Fig.14:3D Response Surface Plots for Viscosity.

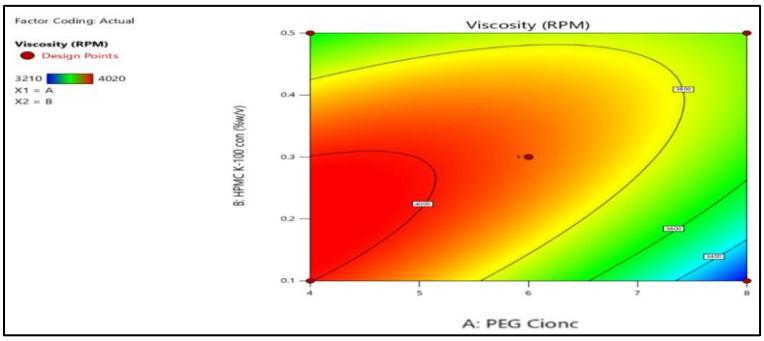

Fig.15: Counter Plots for Viscosity.

From the above graph we can observe that , as conc. of polymer increases, viscosity also increases, it may be due to the more viscous nature of polymer get swelled and hence viscosity get increases.

Permeability study

Formulation no.	Permeability[µg/cm²/h]	Formulation no.	Permeability[µg/cm²/h]
1	56.61±0.032	8	92.24±0.003
2	97.23±0.008	9	92.24±0.003
3	90.77±0.010	10	60.56±0.028
4	94.70±0.006	11	80.78±0.012
5	92.24±0.004	12	92.24±0.003
6	75.12±0.022	13	92.24±0.003
7	78.38±0.019		

Where, n=3, ±SD

Table 10 . In vitro permeability calculations of In- situ nasal gel.

FTIR of Formulation

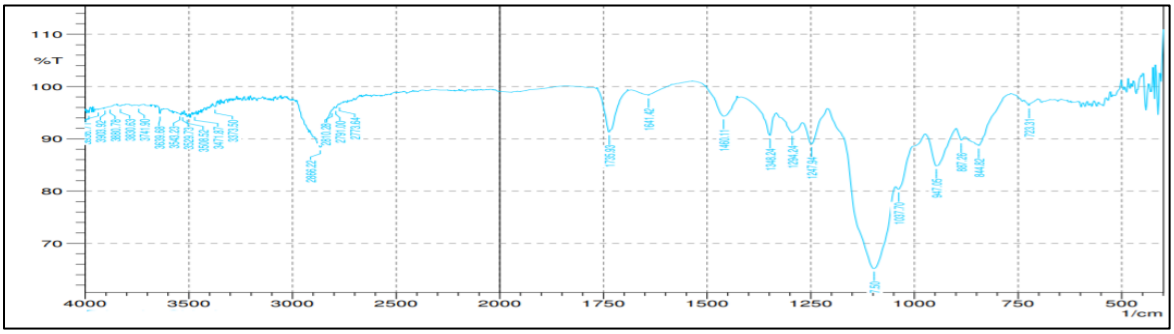

Fig.16: FTIR of Formulation.

The characteristic peaks of such as N-H stretching, C-H stretching, and –C=N ring stretching groups of drug and excipients indicating absence of any interactions between both drugs and drugs with excipients.

Differential Scanning calorimetry (DSC) of formulation

DSC study was performed to observe the thermal properties and intermolecular reaction between the drug Lopinavir and excipients used in the formulation of the polymeric micelles. Pure drug Tranylcypromine showed endothermic peak at 225ºC[19]that corresponds to its melting point. In this DSC thermogram of optimized formulation of polymeric micelle the endothermic peak shifted between100.03 ºC. This might attribute that the drug must be completely entrapped into the formulation.

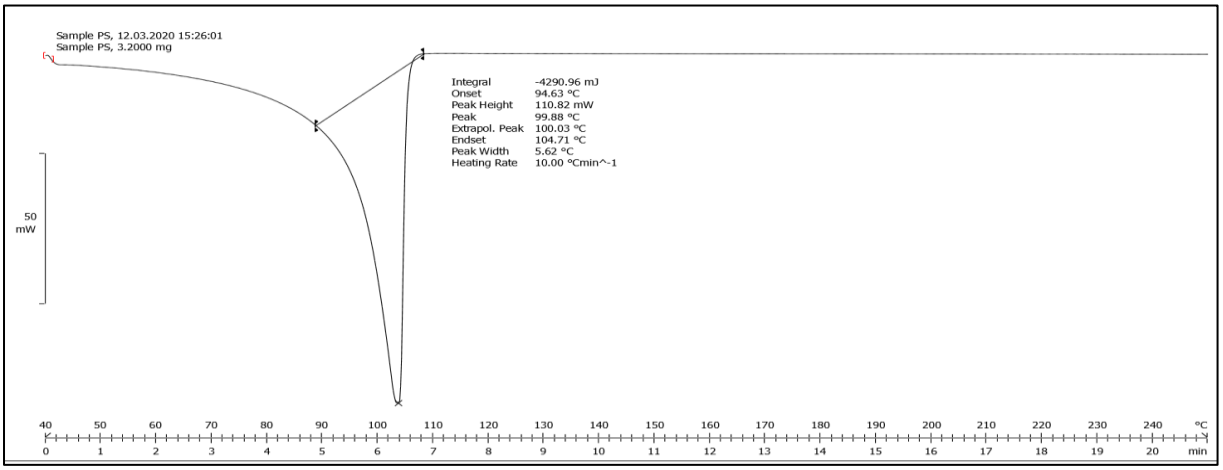

Fig.17: DSC of Formulation.

IV. Conclusion

The study was designed to improve the patient compliance as well as enhancing the bioavailability of drug by directly delivering the drug to the brain by avoiding first pass effect.

In this different polymeric micelles formulation were formulated by using Design Expert applying response surface methodology. Various process variables and formulation variables were evaluated to optimize the formulation via % drug release, % loading as a response to these variables. The optimized formulation showed maximum desirable response as per design expert.

Further, the prepared polymeric micelles of f8 having permeability 95.32 µg/cm^2/hwere successfully loaded in *in-situ* gel which had the desired properties with respect to their clarity, appearance, uniformity and consistency at nasal pH. Drug permeation studies indicate that the effect of drug was prolonged

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