CHAPTER 4

Development, Optimization and Characterization of Micelle Formulation

4.1 Introduction

Nicergoline is poorly soluble slightly yellow coloured powder insoluble in water, soluble in acetic acid or acetone and slightly soluble in ether, chloroform and benzene. Presently in the market nicergoline is present in form of tablet, slowly-releasing granule, capsule, powder-injection and injection. Oral bioavailability of nicergoline is low. Hence, nasal administration of nicergoline will improve its bioavailability as it bypass first pass metabolism, reduce dose and thereby its associated side effects. In comparison to injectable route of administration nasal administration is simple, patient friendly and convenient. As nicergoline acts directly with the brain receptors its intranasal dosage form will carry the nicergoline easily to brain. The nasal cavity is used as the method of administration equivalent to systemic therapy from last few decades. Because there is unique contact between the olfactory lobe and the brain, it's an effective way for brain targeting. The nasal administration of drug will become an effective method that replaces long-term injection.

In the recent years research is focused on intranasal administration of drug. Unstable drugs such as protein and polypeptide, non-absorbable drugs such as antibiotic (e.g. Gentamicin, Cephazolines) are of choice for nasal administration. The intranasal dosage forms have captured a huge market and the nasal delivery system of some of medicine has been patented in lot of countries.

The nasal cavity has area of 150 cm^2 with 15 ml volume. It is covered with 2 to 5mm thick mucous membrane with numerous cilia. The major absorptive area for multiple medicines is the anterior mucous membrane of nasal cavity. The proteolytic enzyme in the mucus layer is one of key factor that affects nasal absorption of medicine. The pH of nasal mucosa is 5.5 to 6.5.At this pH, proteolytic enzymes are active, and if any formulation disrupts the mucus pH value, the activity of proteolytic enzyme is suppressed and the possibility of bacterial infection also get increased. Another mucosa in nasal cavity is olfactory nerve epithelium mucosa. It is connected to central nervous system and is the weakest link of external contact. Its barrier capacity is much less as compared to blood brain barrier. Hence, drugs which are unable to cross the BBB can easily pass through olfactory route and reach the brain target.

Advantages of intranasal administration:

1. Drug can act locally as well as systemically or reach brain target.
2. Drug absorbs rapidly, and take effect time is fast after dosing.
3. It avoids first pass metabolism.
4. Polar and small molecules can be easily absorbed through the nasal mucosa. Absorption of large molecular weight drugs such as polypeptide can also be improved using absorption enhancers.
5. Such route also provide easy to use, high patient compliance, children friendly and can be used for medicines which are required to be taken for long term.

After nasal administration, drug with molecular weight less than 1000 is mainly absorbed by the passive absorption process through the mucous membrane. Bio-availability of fat-soluble medicine after nasal administration is generally similar to that of intravenous administration. The olfactory nerve route is the main route for virus to enter into the brain. Virus is absorbed through the olfactory nerve, transports to the olfactory bulb along with the axoplasma to rat cortical neuron and then reaches rhinencephalon. Nasal route is accepted for most of micro molecules. With the help of pinocytosis or diffusion process, the drug molecule pass through the mucous epithelium olfactory nerve unit, gets into supporting cell, gland cell and gets into the intercellular fluid.

Absorption of drugs through mucous membrane is influenced by physiological factors and physicochemical parameters of drug:

1. Mucous membrane: includes thickness of lipid bilayer, water-soluble space between cells, mucous membrane etc.
2. The physicochemical property of medicine including fat solubility, molecular weight, electric charge, pH and osmotic pressure if medicine.
3. Fat-soluble drugs are easily absorbed through mucous membrane. The hydrophilic and high molecular weight drugs are difficult to absorb. Drugs having molecular weight less than 300 Da can be absorbed rapidly and their absorption is not affected by any other factor. They are easily absorbed through the water-soluble passageway in the membrane. Drugs having molecular mass less than 1000 are easily absorbed through the nasal mucosa. Absorption of drugs having molecular weight >1000 can be increased using absorption enhancer. Nasal mucocilliary clearance is affected by pH,

osmotic pressure and viscosity of formulation, which in turn extends holdup time of medicine in the nasal cavity and thus improve bioavailability.

4. Formulation and concentration of drug in formulation: increase in concentration of drug in formulation increases its absorption to a certain limit. However, for a certain concentration of drug, the formulation also has a prominent influence on the absorption of drug.
5. For local action in nose use of Nasal Drops are sufficient. But, for systemic therapy position of nasal sprays distribution in nasal cavity exerts a tremendous influence on the nose absorbability and thereby bioavailability. Hence, drugs which are metabolized by the first pass effect, with molecular mass less than 1000 and of high fat solubility are suitable candidates for intranasal administration.

In the market nicergoline is available in the form of nasal powder, tablet, slowly-releasing granule, capsule, powder-injection and injection. Out of these, nasal powder formulation is stable, good brain targeting efficiency, user-friendly, more patient compliance, quick effect and more bioavailability.

Surfactant molecules that possess polar head and a lipophilic tail show a change in physicochemical properties once they are added in solution. The change in properties is related to orientation and association of these molecules. This orientation and association of surfactant molecules forms structure called micelle. Based on the type of liquid (aqueous and non-aqueous) micelles are of two type micelle or reverse micelle. Generally, micelles have size in the range of 10 – 200 nm. An average number of monomers that form one micelle at any given time is called as the aggregation number (1, 2).

Because of typical properties such as smaller size, solubility enhancement of poorly soluble drug, targeting efficiency and ease of preparation, micelles have come out as key pharmaceutical carriers. The size and EPR effect, micelles effectively target certain pathological areas in the body with compromised vasculature such as tumours, infarcts. Targeting can also be achieved by attaching specific ligand or specific antibodies onto their surface. Thus polymeric micelles, as drug carriers, have a promising future (3).

4.2 Materials and Equipment

Table 4.1 List of materials

Nicergoline	Gift sample from IVAX Pharmaceuticals ,Opava-komarov, Czech Republic)
Methanol	Spectrochem Pvt. Ltd, Mumbai, India
Chloroform	Spectrochem Pvt. Ltd, Mumbai, India
Poloxamer	BASF
TPGS	Sigma Aldrich
Purified Water	Prepared in Laboratory by distillation.

Table 4.2 List of equipment

INSTRUMENT	**MANUFACTURER**
Weighing balance	Shimadzu, Japan
UV Visible spectrophotometer	Shimadzu, PHARMASPEC UV 1700, (Japan)
Bath sonicator	Modern industrial corporation, Mumbai
Cooling Centrifuge	Remi Equipment Pvt. Ltd., India
Rotary Flask Evaporator	Rota flask IKA RV 10 Digital, Cole Parmer, (country)
pH Meter	Lab. India Pvt. Ltd., India
Vortex mixer	Spinix, India
Lyophilizer	Heto Dry Winner, Denmark
Differential Scanning Calorimeter	DSC-60, Shimadzu, Japan
FTIR	Bruker, (Japan)
Malvern Zeta Sizer	Malvern Instruments, (USA)
HPLC	Shimadzu, Japan
Magnetic Stirrer	Remi equipment Pvt. Ltd., India

4.3 Method

4.3.1 Selection of Surfactant

Screening of surfactant for micelle formulation was carried out based on entrapment capacity of nicergoline in them. Three surfactants were chosen, namely, TPGS, P188, and P407 based on literature (4-8). Entrapment efficiency of surfactant was studied over a range of 1–10%w/v. Surfactant which could completely entrap 4 mg/mL of nicergoline was used for further experiment. The surfactant micelles were prepared by thin film hydration method. Briefly, various amounts of surfactant were weighed and dissolved in 4 mL of chloroform: methanol 1:1 mixture. In case of drug-loaded surfactant micelles, 4 mg of nicergoline was

added in chloroform:methanol containing surfactant. Subsequently, a thin film is formed in round bottom flask using rotary evaporator. Afterwards, film was hydrated with purified water and then centrifuged at 5000 rpm for 15 min to remove any precipitate. Finally, the supernatant was collected for further analysis.

4.3.2Preparation of Nicergoline Loaded Micelles

The drug and surfactants were mixed in the various molar ratios as shown in table 4.7 and dissolved in mixture of chloroform: methanol in round bottom flask. The solvent mixture was allowed to evaporate under vacuum with rotation to form dry thin film and was kept overnight in desiccators to organic solvents, if any. The prepared dry thin film was then hydrated using aqueous hydration medium (Water, Phosphate buffer saline pH 6.8, HEPES buffer pH 8.0).

4.3.3 Separation of Un-entrapped Drug

The un-entrapped drug was removed from the micelle formulation by centrifugation at 4000 rpm for 10 min at 4°C temperature. The supernatant was analyzed for drug entrapment. Sediment was dissolved and analyzed for un-entrapped drug content. The mass balance was confirmed in each case. After analysis the supernatant was filled and stored in amber coloured vial, sealed and stored in refrigerator until required for further experiments.

As entrapment of drug in micelles formed by thin film hydration is dependent upon the process parameters such as hydration medium, volume of hydration medium, ratio of organic solvent, rotation speed of round bottom flask, vacuum applied during thin film formation. These were studied and optimized before optimization of formulation components.

4.3.4 Optimization of Process TFH Parameters

Process parameters also have an impact on the entrapment and size of micelle formulation. Hence following parameters were studied to check their effects.

Table 4.3 Process parameters for micelle formation

Sr No	Process parameter	Level 1	Level 2	Level 3
1	Speed of rotation of RBF (rpm)	100	130	150
2	Hydration Volume (ml)	0.5	1	2
3	Hydration time (min)	15	30	45
4	Hydration Temperature (^{o}C)	60	80	100
5	Vacuum (PSI)	300	400	500
6	Ratio of Chloroform:Methanol	1:0	1:1	0:1

4.4 Optimization of Formulation Components

Pharmaceutical formulations are effected by a number of variables. It is difficult to assess effect of the variables individually or in combination. Factorial designs allow all the factors to be varied simultaneously, thus enabling evaluation of the effects of each variable at each level and showing interrelationship among them. Factorial designs are of choice when simultaneous determination of the effects of several factors and their interactions on response parameters is required. A prior knowledge and understanding of process and process variables under investigation are necessary for achieving a more realistic model. Initial experiments revealed the critical role of polymer concentration (ratio of surfactant and drug) as major variables in determining the percentage drug entrapment efficiency (%EE) and particle size (PS) (**Table 4.4**).

Hence, concentration of TPGS, concentration of PF 127 and concentration of drug were selected as independent variables to find the optimized condition for response variables like maximum entrapment and minimum particle size (PS), so that after peptide conjugation the PS of micelle resides below 30 nm with highest percentage entrapment efficiency (%EE) using Box-Behnken Design with 5 center points and contour plots. Coded and actual values used in optimization are tabulated here (**Table 4.5**)

Table 4.4 Various factors and responses used in optimization

Factors	Concentration of PF (Pluronic F127)
	Concentration of TP (TPGS)
	Concentration of D (Nicergoline)
Response Parameters	%DE (% Drug entrapment)
	Particle size (nm)

Table 4.5 Coded and actual values of process parameters

Coded value	**Actual value**		
	PF (Pluronic F 127) (mg/mL)	TP (TPGS) (mg/mL)	D (Drug) (mg/mL)
-1	10	10	3
0	20	17.5	4
1	30	25	5

Seventeen batches of micelle were prepared by thin film hydration method according to the Box-Behnken Design shown in Table 4.6. Prepared batches were evaluated for particle size, drug entrapment efficiency and the results were recorded (Table 4.7). RSM was applied using comprehensive software, Design-Expert 8.0.4.1 (Stat-Ease Inc., MN, USA) to fit second order polynomial equations, obtained by multiple linear regression analysis (MLRA) approach. Full and reduced model for all variables were established by putting the values of regression coefficients in polynomial equation. Statistical soundness of the polynomial equations was established on the basis of ANOVA statistics (9-15).

Three dimensional response surface plots were established by varying levels of two factors and keeping the third factor at fixed levels at a time (16-18). In this way they are more helpful in understanding the actual interaction amongst the varying factors on the response parameter and are more meaningful. 3-D response surface graphs were constructed using the Design Expert software.

The experimental design and derived polynomial equation for the optimization of formulation were validated for their utility by performing check point analysis. Statistical comparison between the predicted values and average of three experimental values of the response parameters was performed to evaluate significant difference between these values.

Optimized formulation was derived by specifying goals and importance to the formulation variables and response parameters. Results obtained from the software are further verified by actually performing the experiment and comparing the predicted and actual results.

Applied Box-Behnken design matrix is shown in the table along with the responses. The experiments were performed in a random order (in the sequence provided in “Run” column) and responses were recorded.

Table 4.6 Design matrix

Std	Run	Factor 1 A:PF (mg/mL)	Factor 2 B:TP (mg/mL)	Factor 3 C:D (mg/mL)	Response 1 %DE	Response 2 Particle size (nm)
14	1	20.00	17.50	4.00	64	22.9
10	2	20.00	25.00	3.00	87.76	23.7
15	3	20.00	17.50	4.00	72.60	21.8
9	4	20.00	10.00	3.00	85.36	19.6
17	5	20.00	17.50	4.00	69.60	22.2
6	6	30.00	17.50	3.00	62.30	18.9
4	7	30.00	25.00	4.00	82.35	21.8
1	8	10.00	10.00	4.00	80.65	15.6
7	9	10.00	17.50	5.00	79.58	19.5
12	10	20.00	25.00	5.00	83.25	25.5
16	11	20.00	17.50	4.00	79.85	22.3
13	12	20.00	17.50	4.00	95.07	22.4
3	13	10.00	25.00	4.00	74.63	22.3
2	14	30.00	10.00	4.00	69.50	18.3
8	15	30.00	17.50	5.00	79.60	20.5
5	16	10.00	17.50	3.00	72.50	18.9
11	17	20.00	10.00	5.00	76.60	19.9

Detailed method of preparation of micelle by thin film hydration

Pluronic F127 (0.0016 M) and TPGS (0.033M) were dissolved in 10mL of chloroform: methanol (1:1) to obtain a clear solution. Thin film was produced from this solution by solvent evaporation (Rota evaporator, IKA RV 10 Digital, Cole Parmer) at 40ºC for 20 min under reduced pressure (-600 mmHg). Further to remove residual organic solvent it was dried overnight in vacuum oven at room temperature. The dried film was then hydrated using mixture of 0.2% tartaric acid, 10 ml of10 mM HEPES pH 7.0 and incubated at 40^{o}C for 30 min to obtain a micelle suspension. This suspension was filtered through a 0.22-µm nylon filter (Millipore) to obtain mixed micelles of uniform size. To obtain Nicergoline-loaded mixed micelles, 4 mg of drug was added into film formation step as described above. Thus,

clear Nicergoline loaded micelle solution was obtained. This suspension was centrifuged at 5000 rpm for 10 min and filtered through 0.22 μm nylon filter to remove unincorporated Nicergoline aggregates and to obtain clear micelle solution of uniform size.

4.5 Characterization of Micelle

Micelles of Nicergoline were characterized for the following attributes:-

4.5.1 Particle Size and Zeta Potential Analysis

Mean particle size and size distribution was measured by the dynamic light scattering method using a Particle Size analyzer (Malvern Zetasizer, UK). The analyses were performed with 5 mW He-Ne laser (632.8 nm) at a scattering angle of 173^{o}at 25^{o}C. Each freshly prepared sample was diluted to the appropriate concentration using deionised water. The reported experimental result of each sample was expressed as a mean size ± standard deviation (SD).

4.5.2 Zeta Potential Analysis

The zeta potential of various undiluted liposomal suspensions was measured by micro electrophoresis using Malvern Zetasizer, Nano ZS (Malvern Instruments, UK). The instrument works on the principal of Brownian motion and measure the light by Phase Analysis Light Scattering (PALS). Zeta potential of the undiluted micelle was measured to achieve highest sensitivity, accuracy and resolution of zeta potential.

4.5.3 Drug Loading and Encapsulation Efficiency

Drug loading (DL %) and encapsulation efficiency (EE %) was determined by HPLC. Prior to analysis the micelle suspension was dissolved with acetonitrile by bath sonication and warming. DL% and EE% were calculated using following equations:

$$DL\% = \left(\frac{\text{Weight of the drug in micelles}}{\text{Weight of the feeding polymer and drug}}\right) X\ 100 \quad \left(\frac{\text{micelles}}{\text{ymer and drug}}\right) X\ 100$$

$$EE\% = ((Quantity of drug encapsulated)/(Total\ quantity\ of\ Total\ quantity\ of\ drug\ added)) X\ 10$$

4.5.4 Critical Micelle Concentration (CMC) Determination

CMC of binary mixture of Poloxamer F-127/TPGS was determined using an ultraviolet-visible (UV-Vis) spectroscopy method with iodine as a hydrophobic probe. The KI/I_2standard solution was prepared by dissolving 0.5 g of iodine and 1 g of potassium iodide in 50 mL

deionised water. Poloxamer F-127/TPGS solutions with concentrations ranging from 7 x 10^{-8} to 2.1 x 10^{-4}mM were prepared. To each of the Poloxamer407/TPGS binary mixture (molar ratio20:1) solutions, 25µl KI/I_2 standard solution was added. The mixtures were incubated for 12 hours in a dark room at room temperature. The ultraviolet absorbance of different polymer concentrations was measured at 366 nm using a UV-Vis spectrometer (Shimadzu, PHARMASPEC UV 1700). The experiment was performed in triplicate. The graph of logarithm of total polymer concentration Vs the absorption intensity was plotted. The CMC value corresponded to the concentration of the polymer at which the sharp increase in absorbance was observed(4).

4.5.5 Fourier Transform Infra-Red (FT-IR)

The FTIR of Nicergoline, physical mixture of PF 127/TPGS + Nicergoline, Nicergoline-loaded mixed micelles, and blank mixed micelles were recorded against the background by using a FTIR sampling assembly (Bruker). For each sample, the scans were obtained at a resolution of 4 cm^{-1} in the range of 4000 to 400 cm^{-1}.

4.5.6 Differential Scanning Calorimetric (DSC)

The physical state of nicergoline in micelle was determined by comparing DSC of physical mixture of PF-127/TPGS + Nicergoline, Nicergoline encapsulated in the mixed micelles, Blank mixed micelle and the free Nicergoline. The DSC study was done using DSC 60 under nitrogen gas at a flow rate of 40-100 mL per minute. Then, 5 mg of the sample was heated from 0ºC to 300ºC at a rate of 10ºC per minute.

4.5.7 Morphological Analysis of Micelle

Morphology and particle size of micelle were evaluated using Transmission Electron Microscopy (TEM) on Technai 20 Transmission Electron Microscope (Phillips, Holland). For this 50 µl of sample was taken on the carbon film coated on a copper grid and allowed to air dry. Then it was treated with uranyl acetate for negative staining. After 5 min, the grid was placed in sample probe and inserted in Microscope which was then observed at 200 kV accelerating voltage with suitable magnification between 25x to 75000x.

4.6 In Vitro Diffusion Studies

In order to determine the i*n vitro* release of formulation *in vitro* diffusion is a technique to study transport of drug across nasal membrane. According to Gemmell and Morrison

(19)though the *in vitro* models do not mimic the exact condition in living organs/ tissues and do not guess drug transport across the mucosal membrane, they are useful in accessing the relative drug transport behaviour across the mucosa. Parameters such as flux, and diffusion coefficient can be determined using *in vitro* evaluation techniques. Similar to topical formulations, *in vitro* diffusion studies have to be carried out for nasal delivery systems. This will provide relatively quantitative data for comparison and evaluation of these formulations (20). In this study, all test formulations were accessed for *in vitro* diffusion across the sheep nasal mucosa in triplicate and the parameters were calculated.

A. **Percent Drug Diffused**

The percent drug diffused across the sheep nasal mucosa at predetermined sampling time interval was determined using formula mentioned below.

$$\% \text{ Drug diffused} = \frac{\text{Amount of drug in receptor compartment at t time}}{\text{Amount of drug loaded in the donor compartment}} \times 100$$

B. **Kinetics of Release**

In order to investigate the mechanism of drug release from the formulation, the release rates were integrated into each of the following equation and the regression coefficient was calculated.

i. **Zero order equation**

$$Q = K_0 t$$

Where Q - Amount of drug released at time t - Time in hours
K_0 - Zero order release rate constant

ii. First order equation

$$Q = Q_0 e^{-K1t}$$

Where Q - Amount of drug released at time t - Time in hours
K_1 - First order release rate constant

iii. **Higuchi's equation**

$$Q = KH \times \sqrt{t}$$

Where Q - Amount of drug released at time t - Time in hours
KH - Zero order release rate constant

The order of release was determined by performing the regression over the mean values of percent drug diffused vs. time (for zero order), log percent drug remained to

diffuse vs. time (for first order) and percent drug diffused vs. square root of time (for Higuchi order).

C. **Flux :**

The amount 'M' of material flowing through a unit cross section 'S'of a barrier in unit time 't', is known as the flux, 'J'.

$J = dM / S.dt$

D. **Diffusion coefficient:**

The diffusion coefficient of the drug was calculated using the following equation

$J = D \times C_0 / h$

Where J - Flux

C_0 - Drug concentration in donor compartment

h - Thickness of the membrane

Procedure:

The *in vitro* diffusion study was carried out for Nicergoline solution and optimized micelle formulation and analysed at pH 7.4 and 5.5.

1. pH 5.5 to study release at nasal pH

2. pH 7.4 to study drug release after nasal absorption.

Studies were carried out at 37°C in the dark. A known amount of Nicergoline loaded mixed micelles was dispersed in 4mL phosphate-buffered saline (PBS).4 mg/ml micelle solution was introduced into a pre-swollen dialysis tube (MWCO 12000, Sigma-Aldrich). Both ends were sealed and the dialysis tube was immersed into 100 mL release medium PBS (pH 5.5 or 7.4). The whole assembly was maintained at 37^0C and medium was stirred at 75 rpm.At an interval of 0.5,1,2,3,4,6,12 and 24 hr, 5-mL samples were collected from the release medium and replaced with an equal volume of fresh medium. For comparison, the release of Nicergoline from tartaric acid solution was conducted under the same conditions. The concentration of nicergoline in each sample was measured by HPLC. Study was conducted in triplicate. Sink conditions were maintained in the receptor compartment throughout *in vitro* permeation studies (21, 22).

4.7 Nasal Toxicity Studies

Freshly excised sheep nasal mucosa, except the septum part was collected from the slaughter house in PBS buffer pH 6.4. The membrane was kept in PBS pH 6.4 for 15 min. Sheep nasal mucosa pieces with uniform thickness were mounted on Franz diffusion cells. Mucosa were treated with 0.5 ml of PBS pH 6.4, isopropyl alcohol, blank micelle, Nicergoline loaded micelle for 1 hr as well as 2 hr. 2 hr samples were treated with PBS pH 6.4 for 15 min after 1 hr of treatment followed by additional treatment of respective formulations. After 1 or 2 hr the mucosa were rinsed with PBS pH 6.4 and carried to the pathological laboratory in 10 % formalin for the preparation of pathological slides. Nasal mucosa was fixed in 10 % buffered formalin, routinely processed and embedded in paraffin. Paraffin sections were cut on glass slides and stained with hematoxylin and eosin. The sheep nasal mucosa treated with PBS pH 6.4 and isopropyl alcohol were taken as negative and positive control respectively. Sections were examined under a light microscope, to detect any damage to the mucosa during in-vitro permeation by a pathologist blinded to the study (Majithiya et al, 2006). The figure blow shows the histopathological images of nasal mucosa for phosphate buffer (pH 6.4), IPA, and drug solution and blank micelle formulations after 1hr and 2 hr of treatment.

4.8 Result and Discussion

4.8.1Feasibility Trial

Table 4.6 Results of initial feasibility trails

Ingredients	Ratio w/w	% Entrapment Efficiency	Stability
Poloxamer F 127: Nicergoline	1:0.1	20	Stable as such for 24 hours but Precipitate after 50 times dilution
	2:0.1	25	Stable as such for 24 hours but Precipitate after 50 times dilution
	5:0.1	50	Stable as such for 24 hours, stable after 50 times for 2 hours and then precipitate
TPGS: Nicergoline	1:0.1	26	Stable as such for 24 hours but Precipitate after 50 times dilution
	2:0.1	40	Stable as such for 24 hours but Precipitate after 50 times dilution
	10:0.1	98	Stable after dilution and precipitate after 24 hrs of dilution.
Poloxamer F 127:TPGS: Nicergoline	5:10:1	99	Stable after 50 times dilution

Use of non-ionic surfactant such as Poloxamer F 127 and TPGS alone helped in entrapment of nicergoline to some extent, it was mainly concentration dependent (Table 7). When micelles were prepared using TPGS and PF 127 alone in different w/w ratio, at lower concentration of polymers precipitation of drug was observed after dilution in water. But at higher concentration i.e Poloxamer F 127, 5 mg and nicergoline0.1 mg a stable micelle dispersion (50% entrapment) was formed and got precipitated after 2 hr of dilution and in case of TPGS:Nicergoline with 10:0.1 ratio a stable solution (98% entrapment) was obtained, but it also got precipitated after 24 hrs. In terms of solubility of nicergoline forming micelle it was increased from 5 ppm to 50 ppm in combination with PF 127 and 80 ppm in combination with TPGS (Table 4.7).However combination of Pluronic F127 with TPGS, prohibited the precipitation of drug after dilution (Table 4.2), and remain stable for approximately 7 days. Hence we planned for optimization of process parameters on entrapment as follows.

4.8.2 Impact of Process Parameters on Micelle Formulation

4.8.2.1 Speed of Rotation

Effect of speed of rotation on micelle formulation was studied at 100, 130 and 150 rpm. At 100 rpm it was found that the thickness of film was more and it leads to reduced entrapment and formation of lumps. So in order to have uniform size distribution of micelle one has to sonicate the formulation after hydration. More the thickness of film more will be the time required for hydration, more hydration volume and more temperature. At higher rpm it formed a uniform thin film but at the end of vacuum drying the film was shrinked. At 130 rpm the film was found to be uniform with uniform micelle distribution.

4.8.2.2 Hydration Volume

Effect of Hydration volume on micelle formation was studied at three level 0.5, 1 and 2 ml. when 0.5 ml of volume was used it was not sufficient to hydrate the film and maximum amount was evaporated during hydration leading to formation of viscous micelle suspension. Also that amount was not able to cover the thin film surface. When we used 1 ml of hydration liquid we obtained uniform micelle formulation. 2 ml hydration liquid was equally good in comparison to 1ml hydration liquid but it lead to decrease in Nicergoline per ml of formulation. Hence, we used 1 ml of hydration liquid to obtain 4 mg/ml of Nicergoline formulation.

4.8.2.3 Hydration Time

Hydration is a prerequisite for proper maturation of micelles. Hydration time also depends upon the temperature during hydration as it will allow liquefying the thin film in hydration medium. We carried out hydration for 15, 30 and 45 minutes out of which 30 min time of hydration was found to be good in terms of hydration, particle size and complete mixing of thin film on the wall of RBF. In case of excessive hydration there are chances of drug leakage.

4.8.2.4 Temperature for Removal of Organic Solvent

We utilized three temperatures condition for this study, 30^{o}C, 40^{o}C and 60^{o}C. Based on the observation during experimentation, at 30^{o}C more time was required for removal of organic solvent and formation of film. At 60^{o}C organic solvent was removed quickly but at the end of removal of solvent boiling of solvent occurred leading to shrinked film. The temperature condition for thin film formation should be optimum and it should be selected based on the vacuum applied as vacuum will reduce the surface tension leading to decreased boiling point of solvent.

Temperature for removing organic solvent was optimized at 40ºC**.** At this temperature, uniform thin translucent film was formed and hydration at same temperature gave better drug entrapment. This hydration temperature being nearest to the Tg of the polymer and is necessary for orientation of the micelle and intimate packing of lamellae in the hydration medium for good entrapment of drug in the micelle.

4.8.2.5 Vacuum

550, 600 and 650 psi vacuum were used for drying of film.550 psi vacuum was insufficient to remove complete solvent. It may be difficult to remove last traces of organic solvent from the lipid film in case where sufficient vacuum is not attainable this residual solvent may lead to physical destabilization of micelles by interfering with the co-operative hydrophobic interaction. The vacuum 650 mmHg resulted rapid evaporation of the solvent leading to crystallization of the drug and precipitation of polymer due to its comparatively less solubility in organic solvent mixture leading to poor drug entrapment at this vacuum. At higher vacuum air bubbles were entrapped in the lipid film because of lowered boiling point. The vacuum of 600 mm Hg was found to be optimized considering mixture and percentage of drug entrapment without problems of recrystallization.

4.8.3 Optimisation of components of micelle formulation

4.8.3.1 Percent Drug entrapment

p-values for different models, p-value for lack of fit in the models, Adjusted R^2 value and Predicted R^2 values are shown in the following **Table 4. 8**.

Table 4.7 ANOVA analysis of different models

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	
Linear	0.0002	0.0156	0.7055	0.5672	
2FI	0.1491	0.0211	0.7701	0.5443	
Quadratic	0.0043	0.2177	0.9445	0.7395	Suggested
Cubic	0.2177		0.9645		Aliased

Based on the p values and agreement between adjusted and predicted R^2 values, highest polynomial was chosen for response evaluation and prediction of responses. Here, quadratic model was found to best fit the experimental results. Cubic and higher models were found to be aliased (predicted results for one factor would be confounded by the other factors) and hence were left out (**Table 4. 9**).

Table 4.8 ANOVA table for response surface quadratic model

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob> F	
Model	1125.93	9	125.10	31.23	< 0.0001	Significant
A-PF	16.59	1	16.59	4.14	0.0813	
B-TP	548.80	1	548.80	137.02	< 0.0001	Significant
C-D	312.50	1	312.50	78.02	< 0.0001	Significant
AB	50.84	1	50.84	12.69	0.0092	Significant
AC	16.24	1	16.24	4.05	0.0839	
BC	43.16	1	43.16	10.78	0.0134	Significant
A^2	21.85	1	21.85	5.46	0.0522	
B^2	104.97	1	104.97	26.21	0.0014	Significant
C^2	2.32	1	2.32	0.58	0.4711	
Residual	28.04	7	4.01			
Lack of Fit	17.78	3	5.93	2.31	0.2177	Non significant
Pure Error	10.25	4	2.56			
Cor Total	1153.96	16				

Table 4.9shows the summary of ANOVA analysis of chosen quadratic model. The Model F-value of 31.23 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate

model terms are significant. In this case B, C, AB, BC, and B^2 are significant model terms. Concentration of TPGS and drug have significant impact on enntrapment efficiency of drug, while two factor interactions AB and BCalso had significant effect. Concentration of Pluronic F127 and TPGS had quadratic effect on the entrapment efficiency while concentration of drug had linear effect on entrapment. The Lack of Fit F-value of 2.31 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit shows that selected model is appropriate.

Table 4.9 Summary of ANOVA results of quadratic model

Parameter	**Value**
Std. Dev.	2.00
Mean	77.36
C.V. %	2.59
PRESS	300.55
R-Squared	0.9757
Adj R-Squared	0.9445
Pred R-Squared	0.7395
Adeq Precision	19.388

Table 4.10 shows summary of ANOVA results for selected quadratic model. As it can be seen, the "Pred R-Squared" of 0.7395 is in reasonable agreement with the "Adj R-Squared" of 0.9445 implying adequecy of selected model in predicting responses. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 19.388 for quadratic model indicates an adequate signal. Hence, selected quadratic model can be used to navigate the design space.

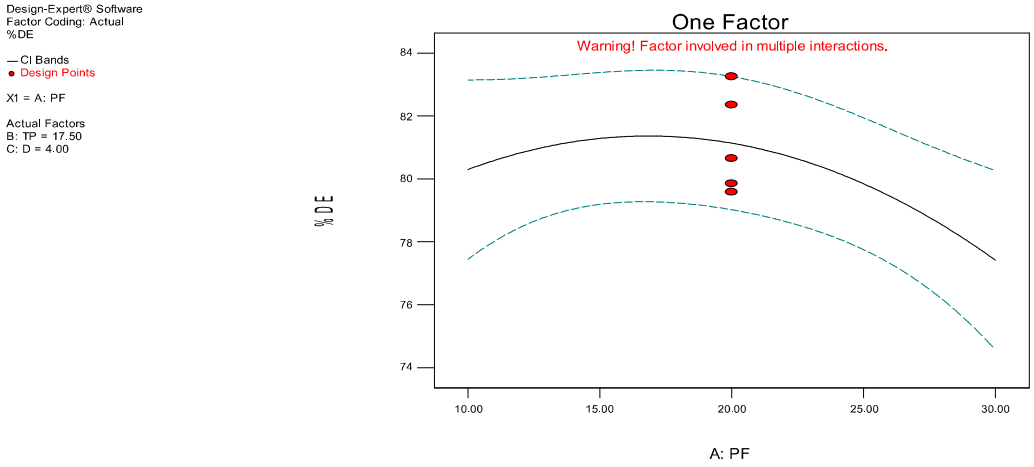

Figure 4.1 Impact of poloxamer F127 concentration on drug entrapment.

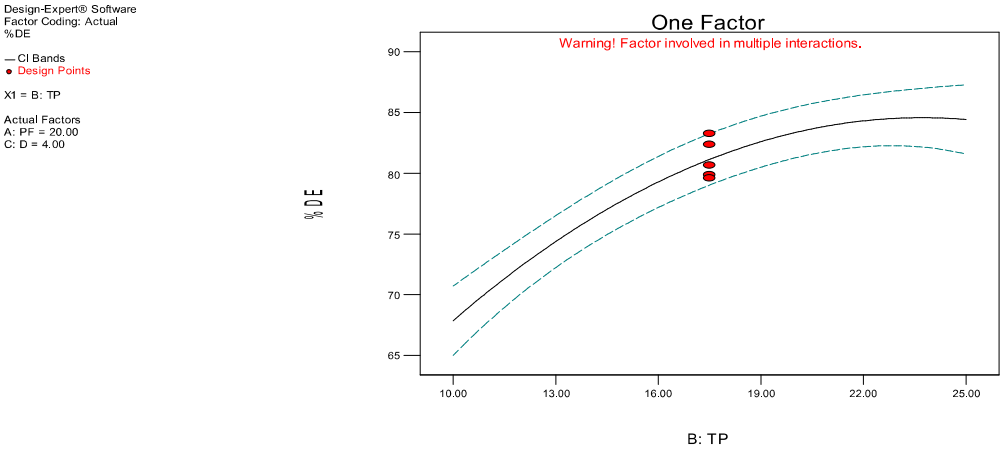

Figure 4.2 Impact of TPGS concentration on drug entrapment.

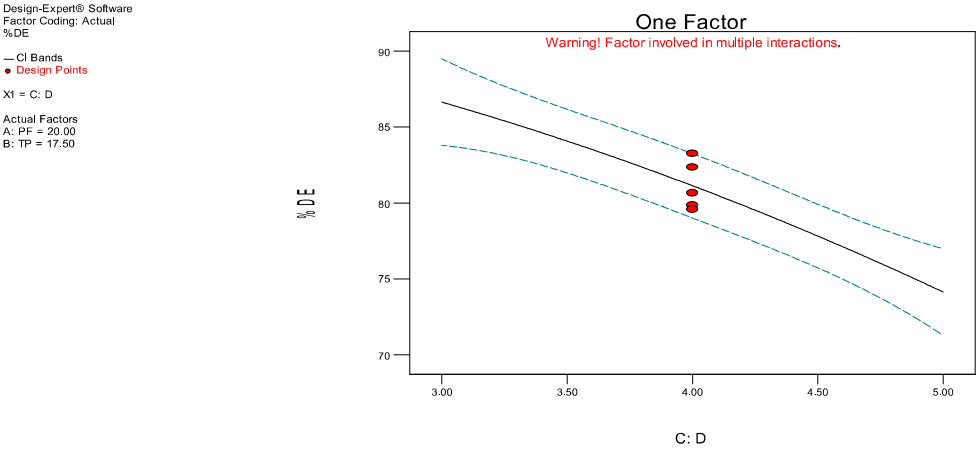

Figure 4.3 Impact of drug concentration on drug entrapment.

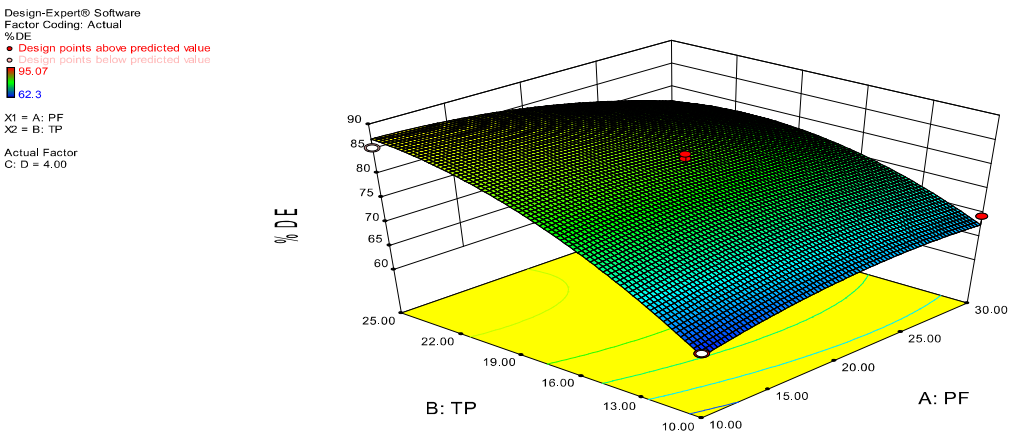

Figure 4.4 Combined impact of PF and TPGS concentration on drug entrapment.

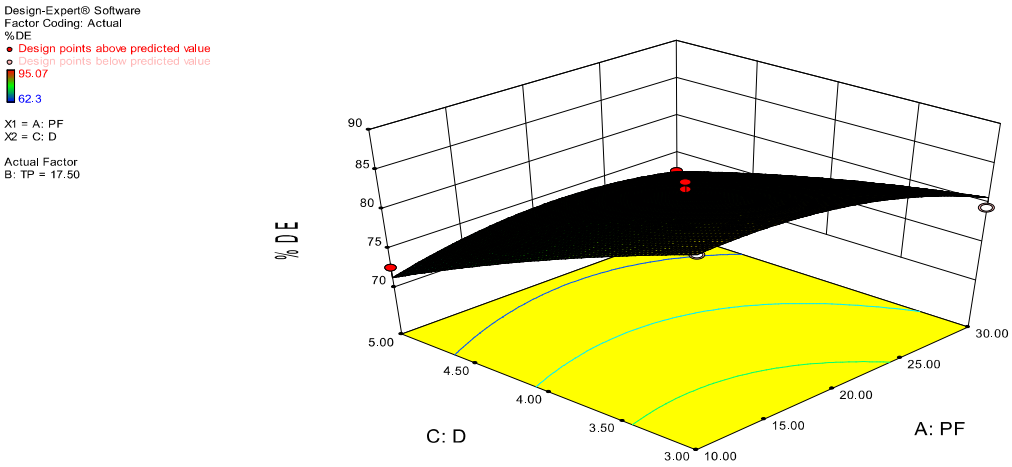

Figure 4.5 Combined impact of PF and nicergoline concentration on drug entrapment.

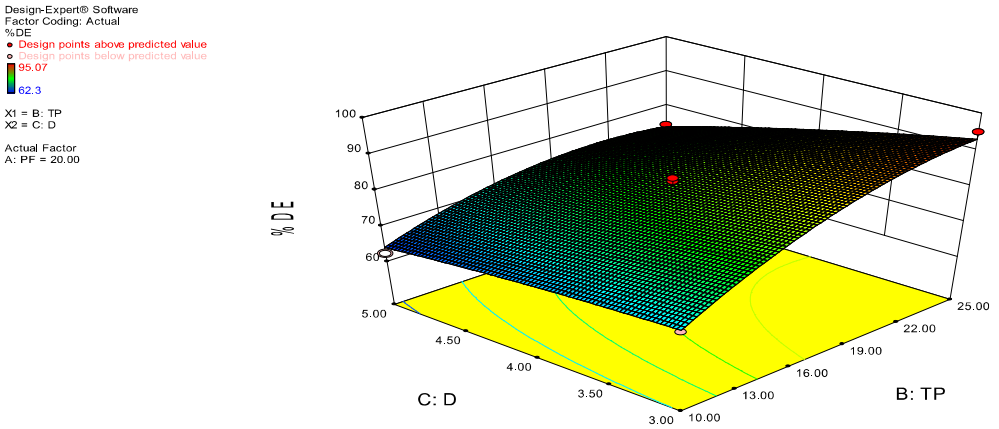

Figure 4.6 Combined impact of TPGS and drug concentration on drug entrapment.

Figure 4.1, Figure 4.2, Figure 4.3 shows one factor effect plots while **Figure 4.4, Figure 4.5, Figure 4.6** show two factor response surface plots depicting the effects of interaction. The major factors which influenced the DL% and EE% of copolymer micelles were the nature and concentration of the solute, nature of the core forming block, core block length, and the nature and block length of outer shell of for micelle. Based on these reasons copolymer carriers composed of PF 127 and TPGS were studied. Concentration of Pluronic F127 and TPGS had a quadartic effect on entrapment. Initially increase in TPGS concentration showed linear increase with that being constant at later stage. However, in case of Pluronic F127, increase in concentraion showed decline in drug entrapment with slight initial increase. This result might be related to the stable interaction among the aromatic ring in TPGS and hydrophobic part i.e Polyoxyethylene groups in PF 127. Increase in concentration of drug caused a linear decline in the drug entrapment. This can be explained by the fact that at higher drug concentrations, surfactants were unable to entrap the whole amount of drug. TPGS increases the size as well as hydrophobicity of the core because of the organic ring moiety of Vitamin E. Hence, as concentration of TPGS increases entrapment increases. But the TPGS moiety alone is unable to hold the drug in the core as after aperiod of 2-3 days of storage of formulation of TPGS alone showed precipitaiton of drug from core. But in combination with Pluonic F 127 stability was achived. The solubility of Nicergolin in obtained micelle formulation is 3mg/ml, while in water is very poorly soluble. We believe that combinaed interaction between the drug and aPluronic/Vitamin E TPGS mixture stabilize the drug moiety inside the micelle core.Raghavan et al have reported that solubility enhancing polymers may adsorb on the surface of drug molecule and reduces the contact between two drug molecules and their by prevents drug nucleation (23). TPGS and PF 127 forms a barrier between two drug molecules andfroms a steric hinderance between drug molecules (24). Polyoxyethylene (POE) moiety of pluronic stabilize the drug molecule by steric hinderance (25).We hypothetize that hydrophilic and hydrophobic interaction between drug and polymer helps in stabilisation of drug moleule even after dilution (23). Also the blocklength ofthe hydrophobic polypropylene oxide (PPO) segment and hydrophobic nature of tocopherol succinate moiety helps in drug stabilisation.

Predicted response for % drug entrapment at any factor level can be calculated using following equation:

%Drug Entrapment =+10.32939
+0.79303 * PF
+6.91376 * TP
+3.32900 * D
-0.047533 * PF * TP
+0.20150 * PF * D
-0.43800 * TP * D
$-0.022780 * PF^2$
$-0.088764 * TP^2$
$-0.74300 * D^2$

4.8.3.2 Particle size

p-values for different models, p-value for lack of fit in the models, Adjusted R^2 value and Predicted R^2 values are shown in the following **Table 4.10**.

Table 4.10 ANOVA analysis of different models

Source	**Sequential p-value**	**Lack of Fit p-value**	**Adjusted R-Squared**	**Predicted R-Squared**	
Linear	0.0075	0.0034	0.4945	0.2478	
2FI	0.8010	0.0020	0.4027	-0.4708	
Quadratic	≤ 0.0001	0.7304	0.9787	0.9514	Suggested
Cubic	0.7304		0.9721		Aliased

Based on the p values and agreement between adjusted and predicted R^2 values, highest polynomial was chosen for response evaluation and prediction of response. Here, quadratic model was found to best fit the experimental results (**Table 4.12**). Cubic and higher models were found to be aliased (predicted results for one factor would be confounded by the other factors) and hence were left out.

Table 4.11 ANOVA for response surface quadratic model

Source	Squares	Df	Square	Value	Prob> F	
Model	89.26	9	9.92	82.60	< 0.0001	Significant
A-PF	1.28	1	1.28	10.66	0.0138	Significant
B-TP	49.50	1	49.50	412.27	< 0.0001	Significant
C-D	2.31	1	2.31	19.25	0.0032	Significant
AB	2.56	1	2.56	21.32	0.0024	Significant
AC	0.25	1	0.25	2.08	0.1922	
BC	0.56	1	0.56	4.68	0.0672	
A^2	32.37	1	32.37	269.55	< 0.0001	Significant
B^2	9.500E-003	1	9.500E-003	0.079	0.7866	
C^2	0.040	1	0.040	0.33	0.5818	
Residual	0.84	7	0.12			
Lack of Fit	0.21	3	0.071	0.45	0.7304	Non significant
Pure Error	0.63	4	0.16			
Cor Total	90.10	16				

Table 4.12 shows the summary of ANOVA analysis of chosen quadratic model. The Model F-value of 82.60 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB and A^2 are significant model terms. All the three factors have significant impact on particle size of micelles. Two factor interactions AB also had significant effect. Concentration of Pluronic F127 had a quadratic effect on the particle size while concentration of TPGS and drug had linear effect on particle size. The Lack of Fit F-value of 0.45 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit shows that selected model is appropriate.

Table 4.12 Summary of ANOVA results of quadratic model

Parameter	Value
Std. Dev.	0.35
Mean	20.95
C.V. %	1.65
PRESS	4.38
R-Squared	0.9907
Adj R-Squared	0.9787
Pred R-Squared	0.9514
Adeq Precision	36.734

Table 4.13 shows summary of ANOVA results for selected quadratic model. As it can be seen, the "Pred R-Squared" of 0.9514 is in reasonable agreement with the "Adj R-Squared" of 0.9787 implying adequecy of selected model in predicting responses. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Obtained ratio of 36.734 for quadratic model indicates an adequate signal. Hence, selected quadratic can be used to navigate the design space.

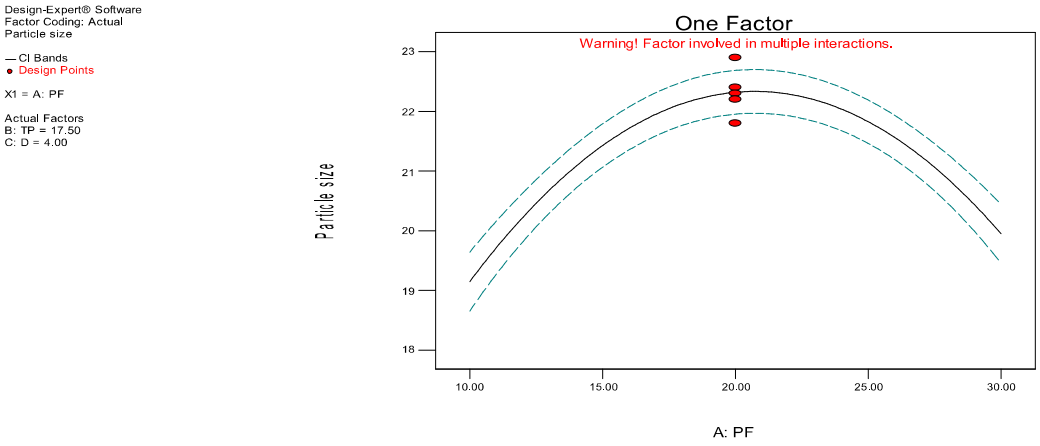

Figure 4.7 Impact of PF on micelle particle size.

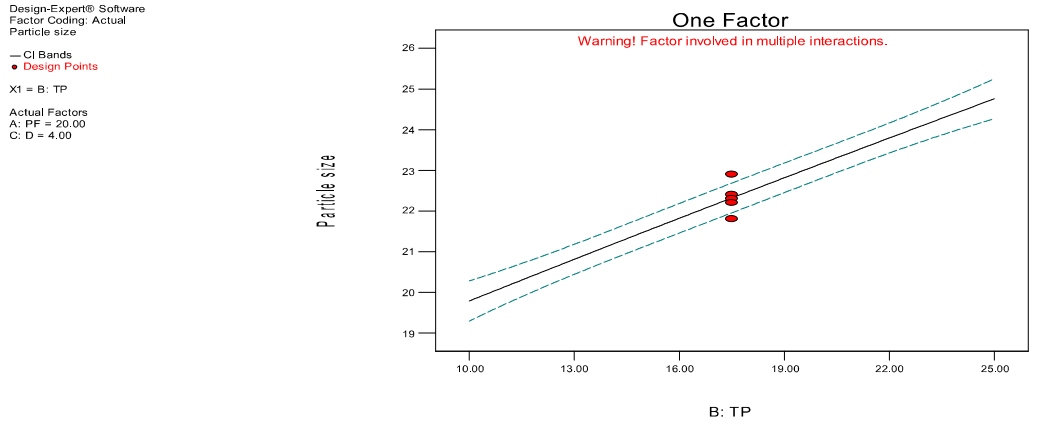

Figure 4.8 Impact of TPGS on micelle particle size.

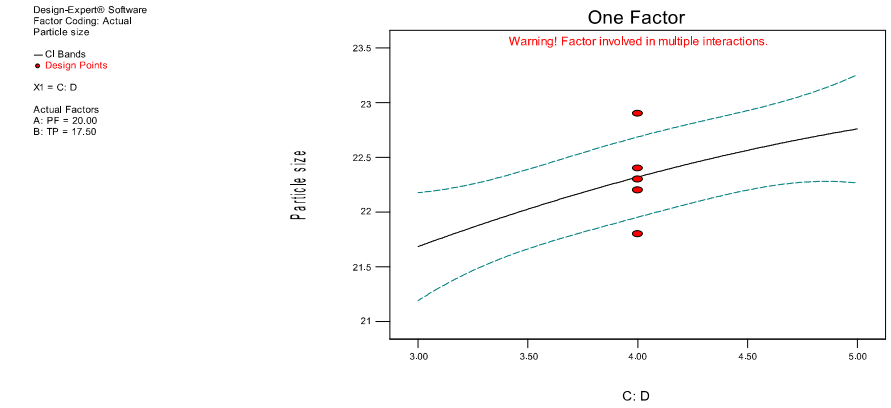

Figure 4.9 Impact of drug on micelle particle size.

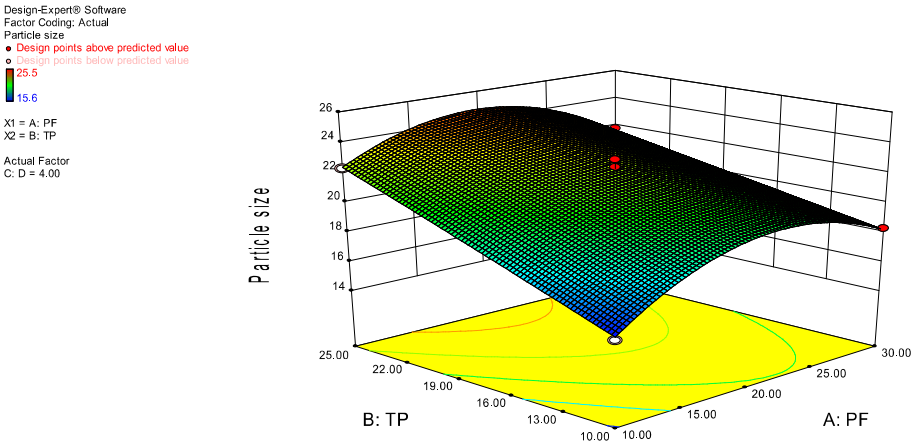

Figure 4.10 Combined impact of PF and TPGS on micelle particle size.

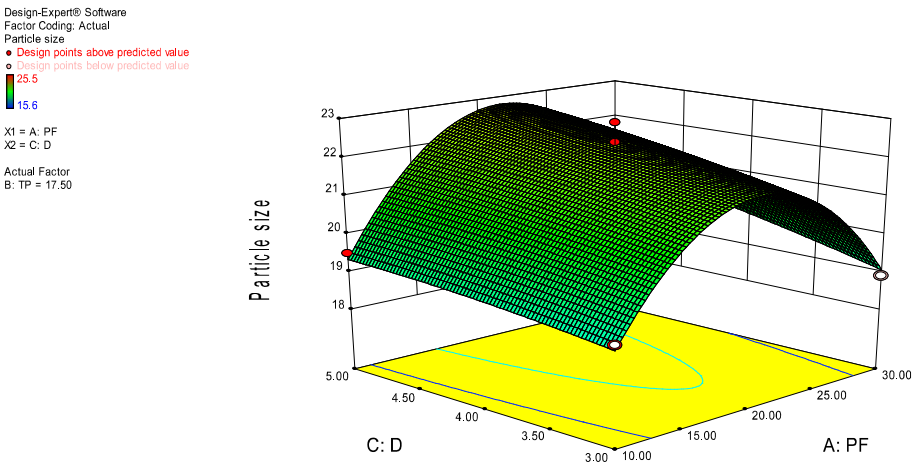

Figure 4.11 Combined impact of PF and nicergoline on micelle particle size.

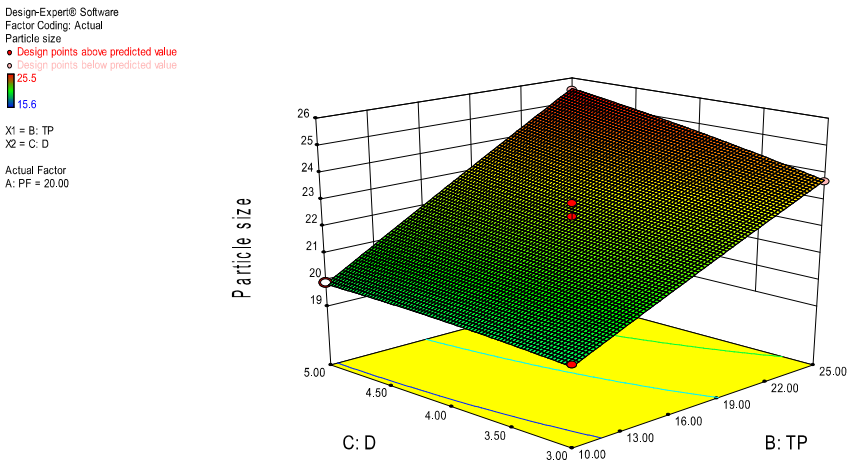

Figure 4.12 Combined impact of TPGS and nicergoline on micelle particle size.

Figure 4.7, Figure 4.8 and Figure 4.9 shows one factor effect plots while **Figure 4.10, Figure 4.11 and Figure 4.12** shows two factor response surface plots depicting the effects of interaction. The size of micelle depends upon the composition of block copolymer, ratio at which two polymers are used and the drug loading. Once the micelle is formed, the micelle size is decided by the hydrophobic interaction between the hydrophobic fractions of polymer and drug.

The size of Nicergoline + PF 127 + TPGS (20.52 nm) was smaller than that of Nicergoline + PF 127 (25.04 nm). This may be due to the impact of polymer composition; the hydrophobic part of TPGS has smaller molecular weight than the PF 127. Hence TPGS easily replaces the Pluronic polymer form the inner core of micelle with smaller diameter, which might result in smaller size (26). Concentration of PF 127 had a quadartic effect on entrapment with a bell shaped curve. Initially increase in PF 127 concentration led to increase in particle size up to around middle level of factor which then led to reduction in particle size. Because initialy at lower concentration PF 127 tries to accommodate with the micelle structure with TPGS. But after middle level of concentration they are seggregated and form their own micelles of smaller size. In case of TPGS and drug, both led to linear rise in the particle size with increase in their concentraion. In case of TPGS and drug, increase in concentration leads to increase in entraptment and increase in core size because it increases the core size.

Stable and small particle size (<200 nm) could reduce the uptake of reticuloendothelial system and provide efficient passive targeting ability via the enhanced permeability and retention effect (27). Therefore the size of prepared micelle was suitable for ischemia specific accumulation with help of targeting moiety.

Predicted response for particle size at any factor level can be calculated using following equation:

Particle size (nm) =+2.42389

+1.23567 * PF

+0.37456 * TP

-0.057500 * D

-0.010667 * PF * TP

+0.025000 * PF * D

+0.050000 * TP * D

-0.027725 * PF2

-8.44444E-004 * TP^2

-0.097500 * D^2

4.8.3.3 Selection of Optimized Formulation

Selection of optimized formulation in terms of concentration of PF 127, TPGS and drug, each dependent factor as well as independent factor (% entrapment efficiency and particle size) were applied with constraints. Constrains were chosen based on the requirement i.e. entrapment efficiency of the formulation was set to be maximized and the importance to the entrapment efficiency was given importance level of 5 (**Table 4.14**). Particle size of formulation was set to be minimized, however importance level was set to 3 as particle size was not so critically affected by factors as compared to entrapment efficiency. Apart from this, slightly higher particle size can be compromised for higher drug loading in the micelles.

Table 4.13 Constraints Applied for Selection of Optimized Batch

Name	Goal	Lower Limit	Upper Limit
A:PF	In range	10	30
B:TP	In range	10	25
C:D	In range	3	5
%DE	Maximize	62.3	95.07
Particle size	Minimize	15.6	25.5

Optimizatoin of formulation was based on the desirability index which may range from 0 to 1 indicating worst fit of the dependent responses in specified goals to best fit of the dependent responses in the specified goals.

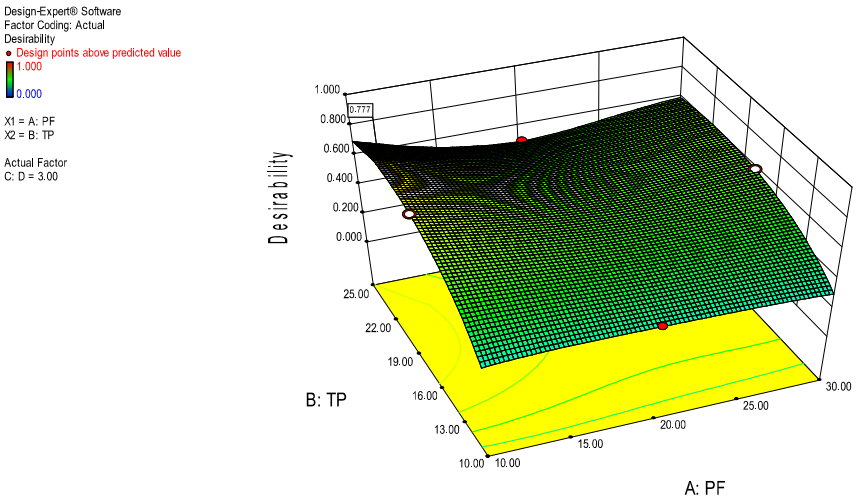

Figure 4.13 Desirability plot for selection of parameters.

Table 4.14 Formulation parameters based on desirability

PF (mg/mL)	TP(mg/mL)	D (mg/mL)	%DE	Particle Size (nm)	Desirability
10.00	21.16	3.00	94.01%	20.17 nm	0.777

Selected formulation parameters Pluronic F127 (10 mg/mL), TPGS (21.16 mg/mL), and drug (3 mg/mL) with desirability of 0.777 were further evaluated for expermimental confirmation. Increased hydrophobic interactions within the core by the presence of the TPGS polymers and poly(propylene oxide) moieties increases entrapment and stability of micelle.

4.8.3.4 Point Prediction and Confirmation

Table below shows predicted response for the selected process parameters along with the Standard deviation and 95 % confidence interval of the responses. Confirmation of the response was done by carrying out the experiment using the selected factor values in triplicate. Results in **Table 4.16** confirm that experimental and predicted values are in good agreement concluding the suitability of the selected models for optimization.

Table 4.15 Experimental confirmation of predicted results

Response	Prediction	Std. Dev.	95% Confidence Interval	Experimental Mean	Std. Dev.
%DE	94.0138	2.00133	89.6438-98.3837	95.254	2.162
Particle size	20.172	0.346513	19.4154-20.9286	19.73	0.49

Table 4.16 Formulation optimization by % drug entrapment

PF-127 (mg)	TPGS (mg)	DRUG (mg)	WATER (ml)	% Drug Entrapment (SD)	% Drug Loading	Physical Stability (1 M)
0	25	4	1	85.62	16.1	Precipitation
10	10	4	1	80.65	12.8	Precipitation
20	10	5	1	76.6	14.5	Precipitation
10	17.5	5	1	79.58	5.9	Crystals formed at bottom
20	25	3	1	87.76	3.9	OK
30	17.5	3	1	62.3	7.0	OK
30	10	4	1	69.5	6.4	OK
10	25	3	1	74.63	6.0	Stable for 90 days in lyophilised form and for 2 M in liquid form
30	25	4	1	82.35	6.8	Precipitation
20	17.5	4	1	64	8.5	OK
20	10	3	1	85.36	7.9	Precipitation
10	17.5	3	1	72.5	12.1	OK
10	0	4	1	30.23	16.1	OK

4.8.4 Micelle Characterisation

4.8.4.1 Particle Size and Zeta Potential

The size of micelle is one of the factors that influence the in vivo distribution of micelle and drug delivery (28). The in vivo extracellular space in rat brain is estimated to be about 38-64 nm at least two folds greater than estimate from fixed tissue. Other nanocarriers such as liposome have been used in size range from 50-150 nm (29). Size of some of the nanocarriers was greater than extracellular space width. Because of shrinkage of extracellular space relatively smaller sized nanocarriers may facilitate interstitial diffusion in ischemic tissue.

Hence, micelle nanocarriers of Nicergoline were developed to exploit their advantages such as low particle size, enhanced permeability across nasal mucosa and ability to incorporate varying ingredients, which would allow targeting of the solubilized Nicergoline, longevity and higher retention effects in the ischemic area, in turn providing an enhanced neuroprotection. From the optimization of formulation we obtained 20 nm micelles with an acceptable polydispersity index of 0.3.

Nicergoline-loaded Poloxamer 407/TPGS mixed micelles were negatively charged with zeta potential of about -13.57 mV. In the structure of Poloxamer 407 both the polypropylene oxide and polyethylene oxide segments were non-ionic, so change of the surface charge of the micelles must have resulted from addition of Nicergoline and/or TPGS.

4.8.4.2 Morphological Analysis of Micelle

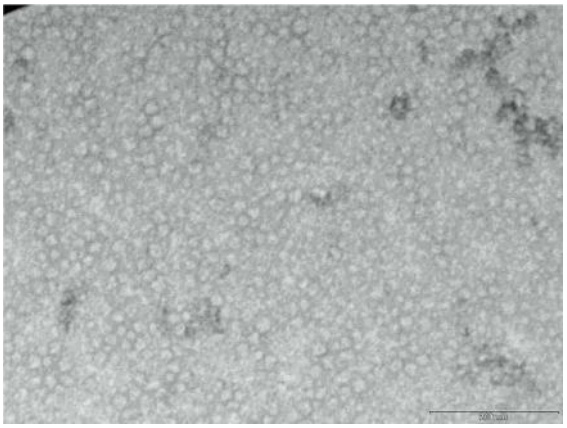

Figure 4.14 TEM images of nicergoline micelle.

TEM micrographs nicergoline-loaded mixed micelles are shown in **Figure 4.14.** The micelles were spherical in shape and uniform in size. The micelle size determined by TEM was similar to that determined by the DLS method.

4.8.4.3 CMC Determination

Pluronic copolymers consist of ethylene oxide (EO) and propylene oxide (PO) blocks, and can undergo self-assembly into spherical micelles in aqueous solution (30).The CMC of polymer influences both in vitro and in vivo stability of formulation. Lower the CMC values of Poloxamer 407/TPGS binary mixture, the higher the stability of Poloxamer 407/TPGS mixed micelles in solutions upon dilution. In this study, CMC of polymer mixture was determined by using iodine (I_2) as a hydrophobic probe. Solubilized 'I_2' participated in the hydrophobic microenvironment of Poloxamer copolymer, thereby causing the conversion of 'I_2' from the excess potassium iodide (KI) in the solution. The graph of absorbance intensity of 'I_2' was plotted against polymer concentration and the CMC of Poloxamer 407/TPGS binary mixture was found to be 7.9×10^{-6} M. The low CMC of the micelles infers to high stability and ability to maintain integrity of the micelles even upon dilution in the blood circulation. The addition of TPGS to micelle formulation did not result in notable variation in the CMC. In previous results it is shown that the CMC of micelle prepared from individual

polymer i.e Pluronic alone and TPGS alone is higher than the micelle prepared from combination of Pluronic and TPGS (31, 32). Therefore, we prepared mixed micelles which weremore stable than pure polymeric micelles. The lower CMC and higher stability of micelle could be explained by the fact that TPGS polymers increase the hydrophobic interactions between the hydrophobic part of Pluronic and the drug in the micellar core and stabilize the structure (27).

Additionally, synergestic interaction between poly(propylene oxide) moieties of Pluronic and the hydrophobic part of TPGS improves the stability of the mixed micelles and lead to incresed core of micelle. Both courses of action hadsignificant impact on the drug solubilization and entrapment efficiency of mixed micelles (33). But the stability of combination is limited to some extent. As the concentration of TPGS was increased, it increased hydrophilic segments i.e succinate-PEG which raise the chances of interaction between hydrophilic and hydrophobic segments and a reduced hydrophobicity of the core subsequently leading to an increased CMC and instability.

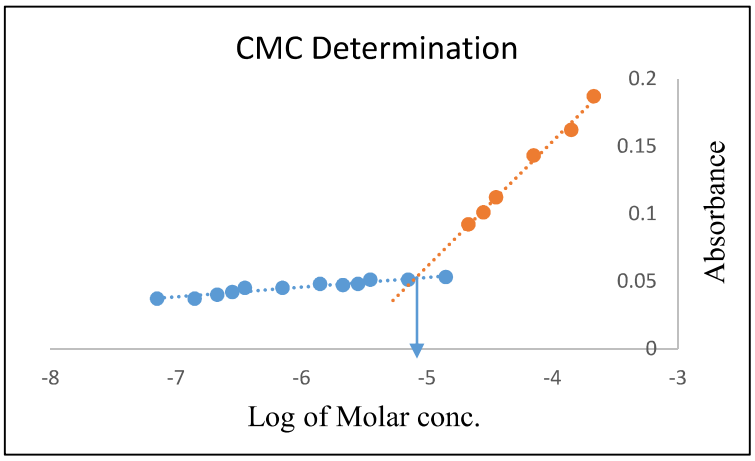

Figure 4.15 CMC determination of micelle

4.8.4.4 DSC Thermogram

The physical status of Nicergoline inside the micelles was examined by DSC. The DSC thermogram of nicergoline, drug loaded micelle, physical mixture and blank micelle are shown in **Figure 4.16**. The thermogram of Nicergoline exhibited sharp endothermic peak at 134.60^{o}C indicated melting point which was reported in literature. Characteristic peak of

Nicergoline was well recognized in the physical mixture. The thermogram of Nicergoline loaded Poloxamer 407/TPGS micelles shows all endothermic peaks of polymers as shown in the blank micelles thermogram, but the characteristic peaks (at 135°C) of Nicergoline was absent, which revealed that nicergoline was molecularly dispersed inside the micelle.

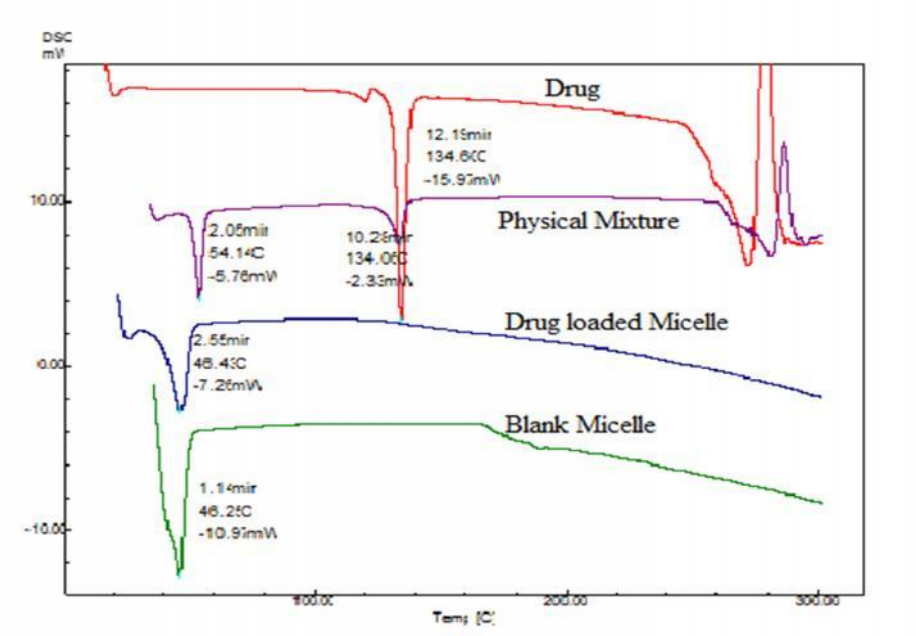

Figure 4.16 DSC Thermogram

4.8.4.5 FTIR

The FTIR spectra of Nicergoline-loaded mixed micelles was compared with those of blank mixed micelles, the physical mixture of PF 127/TPGS + Nicergoline and free Nicergoline in Figure 4.17. IR spectrum of nicergoline exhibited the characteristic bands corresponding to the functional groups of the drug at 3423.99 cm^{-1} characteristic for (- NH stretch) from (3500--3300), at 1720.19 cm^{-1} from (1760--1665 cm^{-1}) characteristic for (C = 0 stretch), at 1081.87 cm^{-1} characteristic for (C--O) and at 1463.71 and 1427.07 cm-1 for (C = C) stretching of aromatic rings. The spectra of the Nicergoline encapsulated mixed micelles showed the absence of characteristic peaks for Nicergoline, which suggested that the drug was localized and entrapped within the hydrophobic core of micelle.

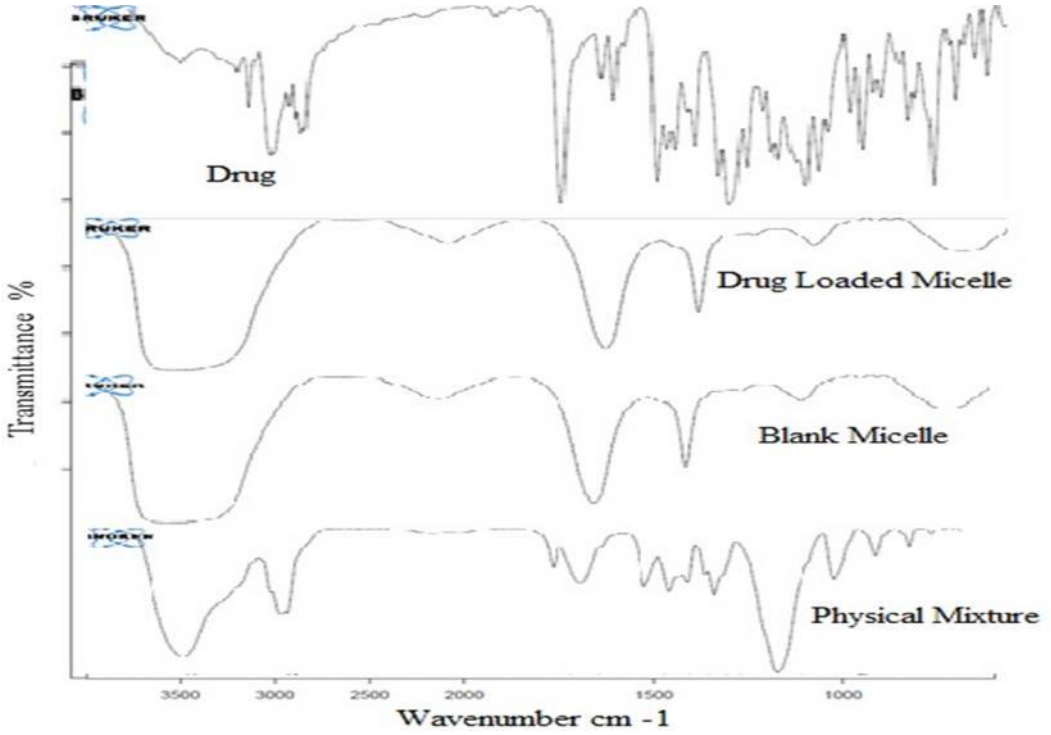

Figure 4.17 FTIR spectra of physical mixture, blank micelle, Nicergoline loaded micelle and Nicergoline.

4.8.5 *In vitro* Diffusion Studies

The *in vitro* release of Nicergoline from micelle formulation under sink condition was investigated by dialysis method with PBS (pH 7.4 and 5.5) as release medium. The percent *in vitro* cumulative drug release of Nicergoline loaded mixed micelle formulation and Nicergoline solution by diffusion method is shown in **Figure 4.18.**

As shown in **Figure 4.18**, Nicergoline release occurred in 2 phases: a phase of burst release followed by a phase of slow and gradual release. Only 45% of Nicergoline was released from Nicergoline –PF 127-TPGS in pH 7.4 and15 % at pH 5.5 within the first 4 h, while almost all Nicergoline was released from the tartaric acid drug solution during the same time period.

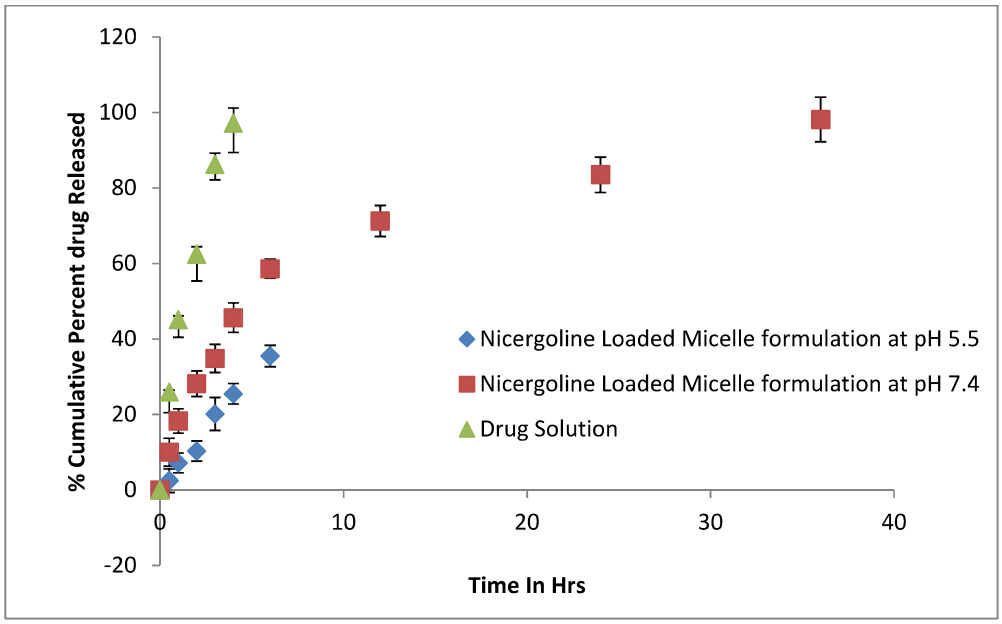

Figure 4.18 *In vitro* diffusion of Nicergoline.

There are two probable reasons for the initial burst release

1. Quick disruption of the micelle system due to cohesion, higher concentration gradient, and sink conditions in the system.

2. A small quantity of nicergoline located at the interface of the micelle hydrophobic core and hydrophilic corona, or even within the micelle corona compartment, releases either by hydration of the interfacial nicergoline molecules and passive diffusion. The nicergoline incorporated into the hydrophobic core remains inside the micelles maintaining a slow release (31).

Two phase release profile reflects the Nicergoline incorporation stability and can be explained through the geometric location of Nicergoline within the micelles. This initial burst release would help in achieving the required therapeutic concentration and the sustained release would maintain the concentration. The lower drug release at pH 5.5 could be beneficial because target for drug action is brain not the nasal mucosa and also it avoids the drug associated side effects. Burst release in pH 7.4 is required as it is physiological pH in brain. However, Nicergoline-loaded mixed micelles could have improved therapeutic efficiency because of their amplified mean residence time in ischemic zone through EPR effects and enhanced drug release in the ischemic tissue.

The micelle carrier not only solubilized the poorly soluble nicergoline, but also sustained the nicergoline release for more than 24 hours. Drug diffusion, polymer erosion or swelling might be possible mechanisms for release of nicergoline from micelles (34). After 24 h, 10-12% of the initially incorporated drug still existed in the micelles at pH 7.4. The result indicated that the micelles showed a sustained-release property for the incorporated Nicergoline, which was similar to the reported studies (27, 35).

Generally drug release form micelle occurs by (36)diffusion through the polymer matrix, release by polymer degradation and solubilisation, or diffusion through microchannels that exist in the polymer matrix. Nicergoline is actually captured in the hydrophobic core of the Poloxamer and TPGS, which controlled its release. Nicergoline from the hydrophobic core is released mainly through diffusion (**Figure 4.18**). As a result, the in vitro release profile of nicergoline from the polymeric micelle system is largely dependent on the interaction with hydrophobic core properties. The strong hydrophobic interaction of Nicergoline with PPO segments of PF 127 and aromatic ring of TPGS may result in a longer diffusion time, leading to a sustained release property.

Table 4.17 *In vitro* drug release kinetics

	pH 5.5		**pH 7.4**	
Drug Release Kinetic	**Line Equation**	**R^2**	**Line Equation**	**R^2**
Zero Order	y = 4.723x - 2.085	0.967	y = 2.245x + 30.2	0.805
First Order	y = -0.001x + 1.999	0.935	y = -0.045x + 1.936	0.975
Hixson-Crowell	y = -0.080x + 4.681	0.957	y = -0.090x + 4.288	0.954
Korsmeyer- Peppas	y = 0.164x - 0.394	0.861	y = 0.491x + 1.313	0.960
Higuchi	y = 11.03x - 5.611	0.798	y = 17.12x + 6.539	0.947

4.8.6 Nasal Toxicity

Optical microscopic images of sheep nasal mucosa treated with IPA, Phosphate buffer (pH 6.4), drug solution, drug loaded micelle formulation and drug loaded peptide conjugated micelle formulations. The prepared formulations were subjected to nasal toxicity to evaluate the safety of the ingredients used in the formulation. The optical microscopy images of nasal mucosa treated with formulations were shown in **Figure 4.19**. The nasal mucosa treated with PBS pH 6.4 showed intact epithelial layer without any damage while mucosa treated with isopropyl alcohol l(mucociliary toxic agent) showed complete destruction of epithelial layer and even deeper tissues

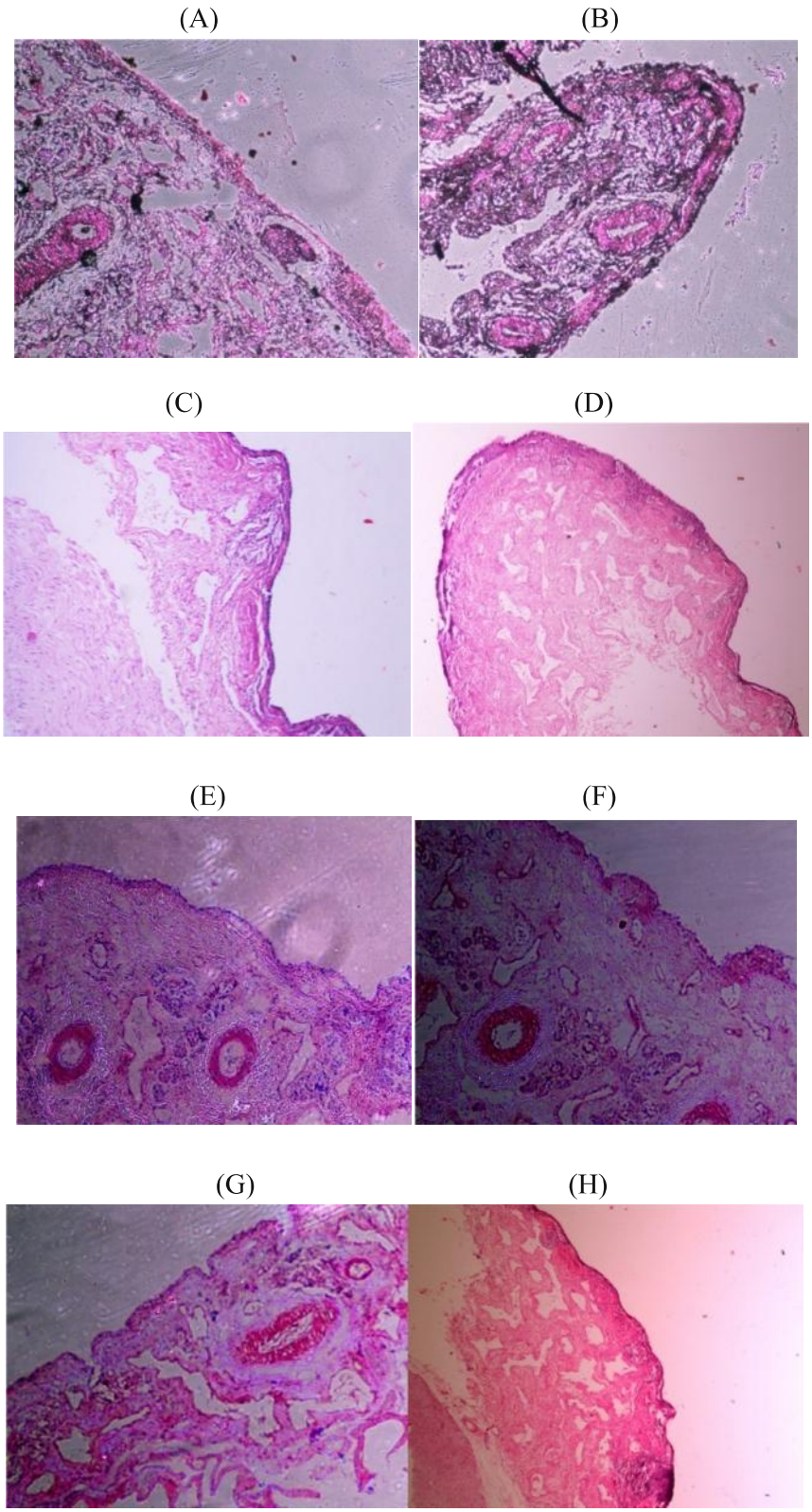

Figure 4.19 Nasal Toxicity in excised sheep nasal mucosa (A) IPA treatment 1 Hr (B) IPA treatment 2 hrs (C) PBS Treatment 1 Hr (D) PBS treatment 2 Hrs (E) Treatment with Drug loaded micelle formulation 1 Hr (F) Drug loaded micelle formulation 2 Hrs (G) Treatment with Peptide conjugated Micelle 1 Hr (H) Treatment with Peptide conjugated Micelle 2 Hrs

Mucosa treated with Blank micelles and drug loaded micelle were found with intact epithelial layer and there were no alterations in basal membrane and superficial part of sub mucosa even after 2 hr of treatment as compared with phosphate buffer (pH 6.4) treated mucosa. This may be due to the lower concentration of Pluronic and TPGS in formulation. Thus, the developed blank micelle and drug loaded micelle seem to be safe with respect to nasal administration.

4.9 Conclusion

This chapter concludes that at lower concentrations of PluronicF127 and TPGS precipitation of nicergoline was observed after dilution with water and a combination of both act synergistically and prepared a stable micelle dispersion whish was stable even after dilution. Less information is available on the mechanism of stabilization of micelle dispersion in combination. The physical characterization such as FTIR, DSC and TEM proved the complete entrapment drug within the micelle of micelle formulation. The *in vitro* release profile also showed the less release of drug at nasal pH and results drug release at physiological pH for longer time. The study results suggest that combination of Pluronic F127 and TPGS is potent combination for nicergoline entrapment. The nasal mucosa treated with isopropyl alcohol (mucociliary toxic agent) showed complete destruction of epithelial layer with no cilia visible while nasal mucosa treated with micelle formulation found to be intact without much damage of the epithelial layer and intact cilia. Thus, the prepared formulations were found to be comparatively safe on nasal mucosa than isopropyl alcohol. However, further toxicity studies need to be conducted prior to clinical application of the prepared formulations.

4.10 Reference

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