A Novel Nasal Fluoxetine loaded Solid Lipid Nanoparticles in Mucoadhesive in situ gel Formulation for brain targeting: Preparation, Characterization and in vivo evaluation

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Abstract:

The blood brain barrier (BBB) poses a significant hurdle to brain drug delivery. However, the location of the olfactory mucosa, within the nasal cavity, is a viable target site for direct nose-to-brain delivery, thereby bypassing the BBB. To exploit this target site innovative nasal formulations are required for targeting and increasing residency within the olfactory mucosa. In the present study, Nasal Gel loaded solid lipid nanoparticles (SLN's) of Fluoxetine were prepared with the objective to provide increased permeability and protection to drug by biocompatible lipidic content and nano-scale size, and thus to develop formulation having potential for enhanced bioavailability and brain targeting.

SLN's of Fluoxetine were prepared by high pressure homogenization technique, emulsification solvent evaporation method and evaluated for particle size (PS), polydispersity index (PDI), zeta potential and entrapment efficiency (EE). Fluoxetine SLN's of average size 101.36 nm, EE 81.56 and Drug release at 24 hr 96.31% were produced.

Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) confirmed the conversion of bulk lipid into SLNs and high entrapment of drug into the lipid matrix. Scanning electron microscopic (SEM) evaluation indicated spherical shape of the formulated solid lipid nanoparticles. The optimized SLN were incorporated in thermo-sensitive in-situ gelling system. The permeation profiles revealed that the drug permeation followed a biphasic pattern with rapid phase for first 2 hr followed by slow phase from 2 to 12 hr. The drug permeation was rapid and more for **FLX -Opt-SLNG1** (9100µg) when compared to the **FLX -Plain Gel** (5083 µg) it was also increased up to 12hr.

Keywords: Fluoxetine, Amoxapine, SLN, High pressure homogenization, solvent evaporation method, *in-situ* gel, *Ex-vivo* permeation studies.

1. INTRODUCTION:

Drug delivery to brain in therapeutic concentrations is still a challenge in spite of massive increase in neurological disorders round the globe. Development of new drug delivery system for brain targeting is complicated due to the presence of blood brain barrier (BBB), comprising of tight layer of endothelial cells surrounded by astrocyte foot processes (1). BBB is a homeostatic defense mechanism of the brain against invading pathogens and toxins. The chief attributes of a drug to cross the BBB are low molecular weight (<500 Da), highly lipophilic. Unfortunately, majority of drugs fail to fulfill these criteria, and cause hindrance in development of brain actives. In fact, approximately 98 % of CNS acting drugs cannot cross the BBB in therapeutic concentration. Therefore, many efforts must be done for the design of strategies to solve this problem.

Nose to brain route is an important and noninvasive method of drug delivery to bypass BBB and avoids the first pass metabolism, it shows enhanced bioavailability of drugs.

Intranasal route of transportation directly delivers the drugs to brain, thus avoiding the side effects and enhancing the efficacy of drug compounds (2). Different strategies have been developed for nose-to-brain drug delivery, that also involve nanomedicine with different kinds of nanocarriers like liposomes, polymeric nanoparticles, microspheres and lipid nanoparticles. Solid lipid nanoparticles (SLNs) offer joint benefits of polymeric nanoparticles, fat emulsions, and liposomes with low toxicity, targeted drug delivery, modulated release delivery, increased bioavailability, good tolerability and biodegradability (3).

Solid lipid nanoparticles (SLNs) are submicron colloidal carriers entrapping drug (either nature- hydrophilic or lipophilic) in a biocompatible lipid core which is either a single lipid or blend of lipids. The only obstacle for drug delivery by an intranasal route is mucociliary clearance, which could be overcome by extending the residence time of the drug in the nasal cavity. The addition of a mucoadhesive polymer that interacts with the nasal mucus layer prevents the clearance of the prepared polymeric delivery system and enhances drug absorption due to prolonged contact with the nasal mucosal surface (4). Drug loaded SLNs will be formulated into in situ gel mucoadhesive formulation, as an appropriate nasal delivery system, to ensure easy intranasal application and enhance its residence time and thus improve its absorption.

2. Materials and Methods

Fluoxetine was purchased from Vamsi Labs Ltd. Maharashtra, India. Glyceryl behenate (Compritol® 85 888 ATO) and glyceryl palmitostearate (Precirol® ATO 5) were kindly provided by Gattefosse India Pvt. Ltd, Mumbai. Glyceryl monostearate, Carbopol 934P was purchased from SD Fine chemicals, Mumbai. Stearic acid, Tween 80 were purchased from SD Fine chemicals, Mumbai. from SD Fine chemicals, Mumbai. Poloxamer 407 from Sigma Aldrich**.** Methanol, Choloroform, ethanol was purchased from SD Fine chemicals, Mumbai.

3 METHODOLOGY

3.1 Identification of Drug Fluoxetine

An identification test is included as an important test to provide an aid in confirming the identity of drug as they are purported and it is based on its appearance, solubility, melting point and confirmed by Fourier-transform infrared (FT-IR) spectroscopic determination (5).

a) Appearance:

The procured sample was visually perceived for its color and was compared with the reported appearance of the drug.

b) Melting point:

Melting point is the identification test method for organic substances. Hence, it is dogged for the sample by capillary method using melting point apparatus.

c) Solubility:

The solubility is determined by "Mechanical shaker method". Quantitative solubility tests are performed as a test for purity. 10 mg sample is taken in a clean dry test tube and solvent is added slowly in aliquots of 0.1 ml with continuous shaking until it dissolved completely. Amount of solvent required for solubilization of the drug powder was recorded.

d) Compatibility studies of Drug

Fourier Transform Infrared (FT-IR) is a significant conforming tool for the solid-state representation of pharmaceutical solids. The sample is made ready by disc method. The drug is mixed with potassium bromide in a mortar-pestle to produce fine and uniform mixture. The pellets are prepared by compacting the powder at 20 psi for 10 min using potassium bromide press. Prepared sample disc is placed in the sample cubicle. Sample is scanned at transmission mode in the region of 4000-400 cm^{-1}. The IR spectrum attained is compared with standard spectrum of pure drug.

3.2 Preformulation studies

The chief components for the Solid lipid Nano particulate system comprises the drug, lipid and surfactant. These selections were based on the solubility of the drug and their ability to produce small sized particles (6).

3.2.1 Selection of Lipid

Different lipids were screened on the basis of solubility studies. The solubility of the drug was determined in different lipids such as Glyceryl monostearate (GMS), compritol ATO 888 (Glyceryl behenate), precirol ATO 5, stearic acid and palmitic acid.

Fluoxetine (20 mg) each was taken in screw capped vial. The solid lipids were separately heated above their melting point. These lipid melts were progressively added in portions to vials containing drugs with continuous stirring using vertex mixer and maintaining the same temperature (above the melting point of lipid).

The end point of the solubility was the formation of a clear, pale yellow solution of molten lipid (7). The lipid viewing maximum solubility for the drug was proposed to have maximum drug loading capacity and was selected for further investigations.

3.2.2 Selection of Surfactant

With the selected lipid, SLN's were prepared using different surfactants and were evaluated for particle size, PDI and entrapment efficiency. The particle size and PDI was determined using Malvern Zetasizer. Selection of the surfactant was made based on minimum particle size and PDI with maximum entrapment efficiency.

3.2.3 Screening of Solvent

In order to determine the solubility of lipid in various solvents, a trial was performed. A known quantity of lipid was slowly added to 2ml of each solvent with continuous shaking. The samples were sealed and stirred for12 hr. The ability of the solvent to dissolve the lipid was

considered when the content looked clear on visual observation (8).

3.3 Selection of formulation Technique

Various technique used for SLN formulation include high shear homogenization, Ultrasound, solvent emulsification and the evaporation technique, microemulsion technique, etc. The technique was selected based on the evaluation of particle size, PDI and entrapment efficiency of the nanoparticles (9).

3.3.1 High Pressure Homogenization (HPH) method

Fluoxetine was added into the melted lipid and the drug loaded lipid phase was dispersed in a hot aqueous surfactant solution under continuous stirring to form a coarse o/w emulsion (10). It was further homogenized at the temperature above the melting point of the lipid using high pressure homogenizer to form o/w Nano emulsion and cooled to a room temperature for solidification and formation of solid lipid nanoparticles.

3.3.2 Emulsification Solvent Evaporation (ESV) Method

Fluoxetine and lipid were dissolved in the mixture of Chloroform and Ethanol. The above organic phase was added as a drop wise to an aqueous solution containing surfactant and co-surfactant (11). The obtained pre-emulsion was subsequently subjected to ultrasonication using probe sonicator to decrease the globules size to the required nanometer range. The formed emulsion was stirred at room temperature using a magnetic stirrer at 400 rpm to allow the organic solvent to evaporate and SLNs to be formed.

3.4 *In vitro* drug release study

I*n vitro* dissolution studies were carried out to assess the release of drug from Fluoxetine SLN formulation, the optimized formulation is compared with the pure drug (12). An accurate amount of aqueous drug suspension, each containing the drug equivalent to 10 mg was packed in dialysis bag andsealed at both ends. The sealed bag was kept in a beaker containing 100 ml of dissolution medium (Phosphate buffer pH 6.4) and stirred at a constant speed at 37±0.5°C. Aliquots were withdrawn at scheduled time intervals up to 24 hr from the receiver compartment and replaced with an equal volume of fresh medium to maintain sink condition. The samples were analyzed spectrophotometrically at λ_{max} of 230 nm.

3.5 Characterization of Optimized Fluoxetine Formulation

Various parameters of Optimized formulation for drug is analyzed by various characterization techniques as follows (13)

3.5.1 Determination of Particle size, Polydispersity index (PDI) and Zeta potential

Average particle size, PDI and Zeta potential were measured by Malvern Zeta sizer

3.5.2 Scanning electron microscopy (SEM) study

Scanning electron microscopy (SEM) was used to study the morphology of the optimized formulation of both drugs. The sample for SEM observation is prepared by lightly sprinkling SLNs dispersion on a double adhesive carbon tape, which was stuck to an aluminum stub. With a help of gold sputter module, the stub was then coated with gold (200 to 500Å thickness) under an argon atmosphere in a high vacuum evaporator. After coating, the sample was

examined under Quanta 200 E SEM scanning electron microscope (magnification: 24000 x; accelerating voltage: 10 KV) at 25±2 ^{0}C.

3.5.3 Differential scanning calorimetry (DSC) analysis

The thermograms of drug, lipid and Optimized Drug-SLNs were recorded with DSC under an inert atmosphere which was maintained by purging with nitrogen. Required amount of the samples (5 mg) was loaded into an aluminum pan and sealed tightly. An empty aluminum pan was used as a reference. Samples were heated at a scanning rate of 10 °C/min over a temperature range between 40–230 °C and the thermograms are recorded.

3.6 Formulation of SLN loaded Fluoxetine Nasal Gel

Carbopol 934 P was selected as gelling and mucoadhesive agents; and Poloxamer 407 as thermosensitive polymers based on literature. Five thermosensitive formulations containing a quantitative amount of the optimized SLNs formulation is prepared using a combination of Poloxamer 407 and Carbopol 934 P by a cold method (14). Briefly, Carbopol 934 (0.5% w/v) and poloxamer 407 (18, 22, 24, 26 and 28% w/v) were gradually added to a cold aqueous solution containing the optimized SLNs formulation (4 °C) over a magnetic stirrer for 60 min until a homogenous dispersion was obtained. The obtained polymeric drug mixture was left in the refrigerator (6 °C) overnight for complete swelling and hydration of the polymers.

3.7 Evaluation of SLN based in-situ gel:

The formulated SLN based gel was evaluated for pH, viscosity, mucoadhesive strength and drug content.

3.7.1 pH: pH of the nasal formulations was determined using pH meter and was compared with the reported pH in nasal cavity. One ml of prepared gels was transferred to 10 ml volumetric flask, and the solution was diluted with distilled water. The pH of the resulting solution was determined using a digital pH meter (15).

3.7.2 Viscosity: Viscosity of the formulations should be such that it remains convenient during their administration by the patient. Viscosity of the formulation was determined at 34°C (nasal temperature) using brookfield viscometer.

3.7.3 Mucoadhesive strength:

The mucoadhesive force was determined using modified two-pan balance method. It is the most common and convenient methods to assess the mucoadhesive properties of formulations. In this method, one side of the balance was provided with blocks at the top for balancing and the other side had a receptacle for water as shown in Figure 1.1. 20 µl gel test sample was applied on the perfectly horizontal surface and was just touched with the cellophane membrane (1 cm^2) stacked at the horizontal end opposite to that of water receptacle. Water is added drop by drop till; the cellophane membrane gets detached from the gel.

Figure 1.1: Modified two-pan balance method

Weight in grams of water required to separate the two surfaces was measured and mucoadhesive force was calculated using (1)

F = w x g ……...…….. Equation 1

Where F is the mucoadhesion force (dynes / cm^2),
w is the minimum weight required to break the bond (grams),
g is the acceleration due to gravity (cm/s^2).

3.7.4 Drug Content Estimation:

Formulation equivalent to 10 mg of the drug was diluted with distilled water and after suitable dilutions the absorbance was measured at 230 nm using UV visible spectrophotometer. The drug concentration was calculated as shown in below. Further, % drug content was calculated from the concentration using the following equation:

$$\% \textbf{ Drug Content} = \frac{\textbf{Concentration of drug in sample solution}}{\textbf{Equivalent concentration of drug taken}} \textbf{ X 100}$$

3.8 Ex *vivo* permeation studies of Optimized Fluoxetine loaded SLN Nasal Gel

The *ex vivo* permeation studies for the Optimized formulation of Fluoxetine loaded gel, and plain gel was carried out by using freshly excised porcine nasal mucosa in PBS pH 6.4. The studies are carried out for 12 hrs by taking the formulation (10mg) in the donor compartment and the PBS pH 6.4 in a receiver compartment of the Franz diffusion cell (16). The cumulative amount of drug permeated, Flux is calculated.

3.9 Stability Study of Optimized Formulation Gel

Stability of optimized gel performed at both Refrigeration temperature (5 ± 2 °C) and 25 °C/60 ± 5 % RH for 6 months according to ICH guidelines. The formulation did not show any caking and phase separation during a whole six-month stability study (17). No significant ($p < 0.05$) variation was found in Drug content %, Viscosity and Mucoadhesive strength.

4. Results & Discussions of Fluoxetine (FLX)

4.1 Identification of Drug Fluoxetine

a) Appearance & Melting point:

Table 1.1: Identification of Drug Fluoxetine

S.No.	Description Attributes of Drug	Inference
1	Colour	White to white pale
2	Melting point	168-178℃

b) Solubility:

Table 1.2: Solubility of Drug Fluoxetine

S.No.	Solvents	Solubility of Fluoxetine at 25^0 C	Concentration(mg/ml)
1	Hexane	Very soluble	380±11.69
2	Ethyl acetate	Freely soluble	360±12.4
3	Methanol	Freely soluble	124±5.2
4	Dimethyl Formamide	Soluble	39.1±0.38
5	Water	Sparingly Soluble	17.5±0.34
6	0.1 N HCI	Slightly Soluble	5.2±.69
7	7.4 Phosphate Buffer	Slightly Soluble	2.4±0.11
8	6.4 Phosphate Buffer	Very Slightly Soluble	1.69±0.21

Solubility of the drug was determined in various solvents at room temperature. These observations showed that the drug is highly lipophilic in nature.

c) Compatibility studies of Drug

From the observations of, it was verified and concluded that the procured sample was of Fluoxetine and the sample was used for all further investigations.

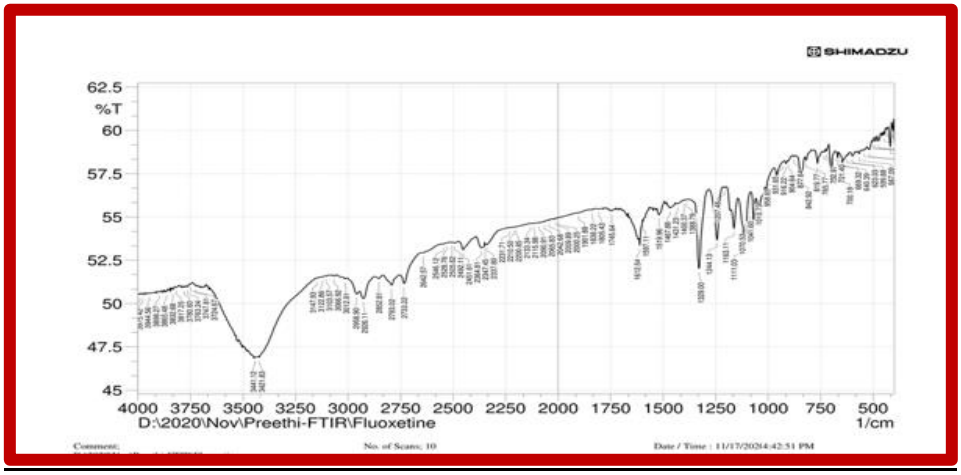

Figure 1.2: FTIR of Fluoxetine Pure drug

IR spectra of Drug (Fluoxetine), lipid (GMS), surfactant (tween 80) and physical mixture of drug, lipid & surfactant were scanned from 4000 cm-^{1} to 400 cm $^{-1}$ & recorded, are shown in Figure 1.2

FTIR spectrum of Fluoxetine showed the characteristic peaks of the drug structure. Important peaks at 3120 cm-^{1} appeared the characteristic peak of OH stretch, at 2951 cm-^{1} aromatic of CH group stretch, at 2911 cm-^{1} of aliphatic CH_2 stretch, at 1676 cm^{-1} and 1587 cm^{-1} corresponded to C=O carbonyl stretching and substituted aromatic ring, respectively, at 1138 cm^{-1}. CH deformation of F substituted aromatic ring, at 995 cm-1 is due to –Cl substituted aromatic ring.

4.2 Preformulation studies

4.2.1 Selection of Lipid

The solubility of the drug was determined in six different lipids - The solubility of the drug was determined different lipids – Glyceryl monostearate (GMS), Glyceryl Compritol 888 ATO, Glyceryl palmitostearate, Precirol ATO 5, Stearic acid and Cetyl palmitate. The results obtained are as shown in Table 1.3

Table 1.3: Solubility of Fluoxetine in Various Lipids

Lipids	Melting point range ^{0}C	Drug solubility in lipid (mg/gm)
Glyceryl Monostearate (GMS)	**59**	**110±8.01**
Precirol ATO 5	56	55±5.01
Cetyl palmitate (CP)	43	51±5.11
Glyceryl palmitostearate (GPS)	53	46±5.01
Compritol 888 ATO (CMP)	70	45±6.04
Stearic acid	69	32±5.01

The solubility of drug in Glyceryl monostearate was found to be significantly higher than other lipids ($p < 0.05$). The ester functional group of GMS induced the negative charge of the formulations and enhanced the stability of the SLN formulation. Glyceryl monostearate showed maximum drug solubilizing capacity of 110±8.01 mg drug / gram of lipid.

4.2.2 Selection of Surfactant

For the selection of surfactant, nanoparticles were prepared with six different surfactants using GMS as lipid and were evaluated for particle size and entrapment efficiency. The results obtained are as shown in Table 1.4.

Table 1.4: Screening of surfactant based on particle size & Entrapment efficiency

S.No.	Lipid	Surfactant	Particle size (nm)	Entrapment Efficiency (%)
1	**GMS**	**Tween 80**	**251±7.04**	**68.21±2.84**
2	GMS	Tween 60	362±9.01	49.23±4.65
3	GMS	Tween 20	432±6.83	40.12±4.21
4	GMS	Span 20	588±7.11	36.54±2.35
5	GMS	Span 60	410±8.84	48.87±6.25
6	GMS	Span 80	350±8.04	37.21±1.32

Tween 80 was found to give minimum particle size and PDI with maximum entrapment efficiency.

4.2.3 Screening of Solvent

Solubility of the drug was determined in various solvents at room temperature. Fluoxetine was found to be freely soluble in chloroform, methanol; slightly soluble in ethanol, acetone.

Table 1.5: Solubility of Fluoxetine in different solvent

Solvent	**Solubility (mg/ml)**
Chloroform	600±12.31
Methanol	512±11.34
Ethanol	311±25.31
Acetone	254±21.03

Table 1.6: Solubility of Fluoxetine in different mixture of solvent

Solvent	**Solubility (mg/ml)**
Chloroform: Ethanol	801±16.14
Chloroform: Methanol	712±15.03
Chloroform : Acetone	611±17.11

Solvent was selected on the basis of solubility of the lipid in the different solvents and solvent mixtures. Out of all, the mixture of chloroform and ethanol (1:1) exhibited the highest solubility.

4.3 Selection of Formulation Technique

Methods of Preparation:

SLN formulations were prepared by different techniques (as trials) and were compared with respect to particle size and entrapment efficiency. The results obtained are as shown in Table 1.7 & 1.8

Table 1.7: Emulsification Solvent Evaporation Method SLN (ESV-SLN)

S.No.	Trial Batch	Particle size (nm)	Entrapment Efficiency (%)
1	ESV-SLN1	211±12.02	68.17±4.17
2	ESV-SLN2	243±11.07	60.21±6.02
3	ESV-SLN3	285±14.12	70.13±4.56
4	ESV-SLN4	156±11.69	69.59±9.14
5	ESV-SLN5	197±14.33	76.93±8.35
6	ESV-SLN6	201±21.02	81.62±7.25
7	ESV-SLN7	255±21.14	79.37±4.25
8	ESV-SLN8	206±22.47	75.82±6.24
9	ESV-SLN9	189±14.11	58.37±8.14

Table 1.8: High Speed Homogenization Method (HSH-SLN)

S.No.	Trial Batch	Particle size(nm)	Entrapment Efficiency (%)
1	HSH-SLN1	299±32.14	40.11±8.55
2	HSH-SLN2	341±22.14	52.14±7.32
3	HSH-SLN3	311±27.31	48.62±4.11
4	HSH-SLN4	399±20.11	39.14±5.68
5	HSH-SLN5	382±25.14	51.24±6.12
6	HSH-SLN6	424±31.25	48.77±9.34
7	HSH-SLN7	461±23.45	38.74±8.11
8	HSH-SLN8	358±32.47	49.14±5.24
9	HSH-SLN9	465±24.07	48.02±4.12

It was observed that lower particle size with higher entrapment efficiency was obtained by Emulsification solvent evaporation method. Based on the evaluation result of Particle size and entrapment efficiency, Emulsification solvent evaporation method was used for further investigation.

Table 1.9: Observed Responses for 15 Runs of FLX- SLNs using Box-Behnken Design

Standard Runs	Formulation Code	Lipid (X1)	Surfactant (X2)	Co-surfactant (X3)	Particle size	EE%	DR% at 24 hr
5	FLX-SLN1	20	10	5	165.43	61.96	89.51
12	FLX-SLN2	20	15	4	186.4	64.28	96.39
13	FLX-SLN3	20	15	6	190.4	71.38	85.34
11	FLX-SLN4	20	20	5	213.65	68.63	84.65
14	FLX-SLN5	30	10	4	125.83	80.12	94.33
9	FLX-SLN6	30	10	6	136.45	80.56	92.42
2	FLX-SLN7	30	15	5	117.65	79.82	95.32
3	FLX-SLN8	30	15	5	101.36	81.56	96.31
6	FLX-SLN9	30	15	5	98.64	80.21	94.45
10	FLX-SLN10	30	20	4	140	76.28	91.36
4	FLX-SLN11	30	20	6	151.54	75.63	90.23
8	FLX-SLN12	40	10	5	239.43	53.56	83.65
15	FLX-SLN13	40	15	4	242.43	55.84	82.51
7	FLX-SLN14	40	15	6	243.76	58.56	81.03
1	FLX-SLN15	40	20	5	254.98	56.85	80.81

4.4 In-*vitro* release study

The dialysis bag method was used to determine the release profile of the drug from SLNs. This is an efficient method to study the drug release from nanocarriers. Release of drug molecules from SLN's usually follows a biphasic pattern characterized by an initial burst release of the loaded drug followed by a phase of sustained drug release.

Table 1.10: *In-vitro* release of Fluoxetine loaded SLN's formulations F1-F15

Time (hr)	FLX-SLN1	FLX-SLN2	FLX-SLN3	FLX-SLN4	FLX-SLN5	FLX-SLN6	FLX-SLN7	FLX-SLN8	FLX-SLN9	FLX-SLN10	FLX-SLN11	FLX-SLN12	FLX-SLN13	FLX-SLN14	FLX-SLN15
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	23.12	22.14	25.36	20.14	28.33	29.14	25.33	24.01	20.14	24.44	26.31	27.22	21.24	26.14	21.32
8	35.14	34.18	34.18	34.18	34.18	31.47	39.24	36.02	37.18	34.22	33.15	36.14	37.27	34.48	30.24
10	65.18	62.31	64.32	63.11	66.31	67.32	68.03	69.13	67.12	65.22	65.23	64.32	62.11	60.31	59.32
12	76.33	72.31	74.33	72.32	81.31	80.14	83.27	84.32	81.47	80.33	79.82	71.03	70.32	71.31	70.14
24	89.51	86.39	85.34	84.65	94.33	92.42	95.32	96.31	94.45	91.36	90.23	83.65	82.51	81.03	80.81

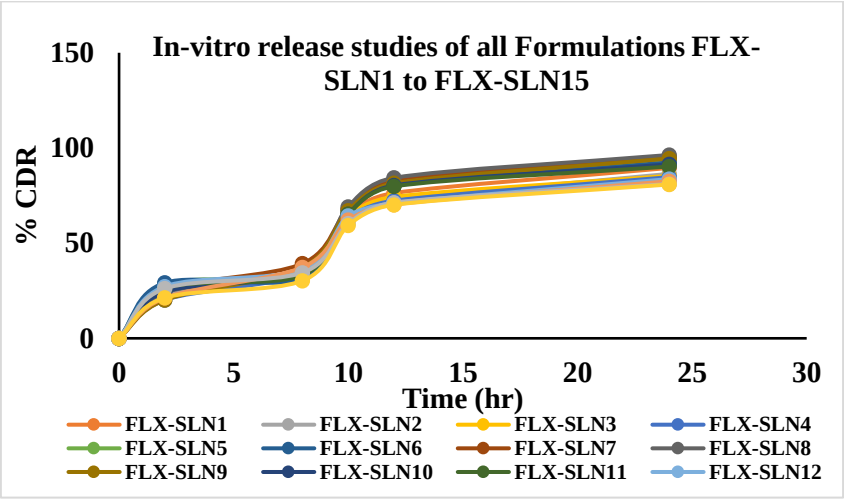

Figure 1.3: *In vitro* release studies of all Formulation (F1-F15)

The initial burst in drug release can be caused by the accumulation of drug in the outer shell of the SLN's due to the phase separation that occurs during lipid crystallization, leading to fast release of the drug from the surface of the particles. In contrast, the sustained release pattern occurs when the drug molecules entrapped in the core of the NPs are slowly released due to the partition between the oil and water phases, diffusion of the drug or erosion of the matrix.

Table 1.11: Comparison of Optimized Formulation with pure dispersion

Time (hr)	FLX-Dis	OPT-FLX-SLN
0	0	0
2	45.12	29.14
8	95.35	31.47
10	---	77.32
12	---	82.14
24	---	96.44

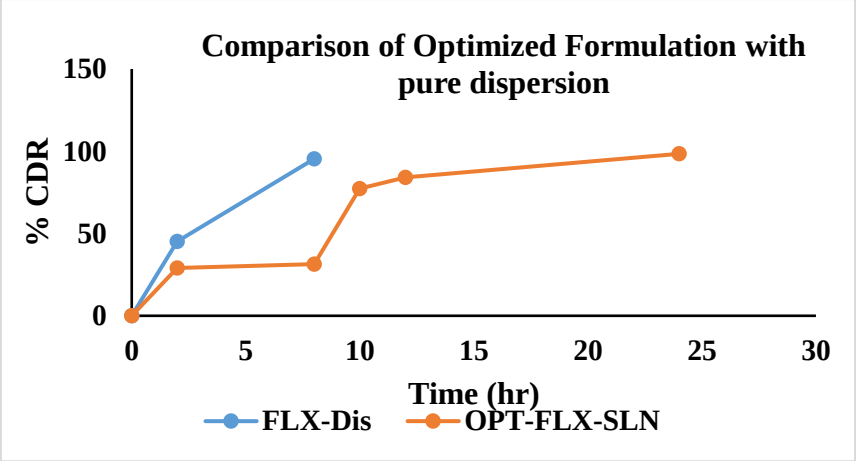

Figure 1.4: Comparison of Optimized Formulation with pure dispersion

The release of drug from the SLN dispersion was found to be more consistent in comparison to the release from plain drug suspension. In-vitro drug release from optimized batch (OPT-FLX-SLN) and pure drug dispersion was carried out under the same conditions and compared (Fig.1.4). Within first 2 hr, the % cumulative drug release from optimized batch was found to be 29.14% indicating the initial burst effect due to presence of absorbed drug onto the surface of SLNs and /or free drug in the external phase followed by sustained/slow release up to 24 hr (96.44%). Slow release from SLNs may be due to the encapsulation of drug with GMS core. Almost the entire drug was released from pure drug dispersion within 8 hr (95.35%) indicating a poor release control. Statistically significant ($P< 0.05$) difference was marked between % cumulative drug release from SLNs and pure drug dispersion.

4.5 Characterization of Optimized Formulation

4.5.1 Drug-Excipient Compatibility Study

IR spectra of Drug (Fluoxetine), lipid (GMS), surfactant (tween 80) and physical mixture of drug, lipid & surfactant were scanned from 4000 cm^{-1} to 400 cm^{-1} & recorded, are shown in Figure 1.5 to 1.7. These characteristic peaks of Fluoxetine were also observed in the FTIR spectrum of physical mixture of Fluoxetine, GMS and tween 80 without any significant distinct shift. This fact verified that no chemical interaction was observed between the drug and selected polymer.

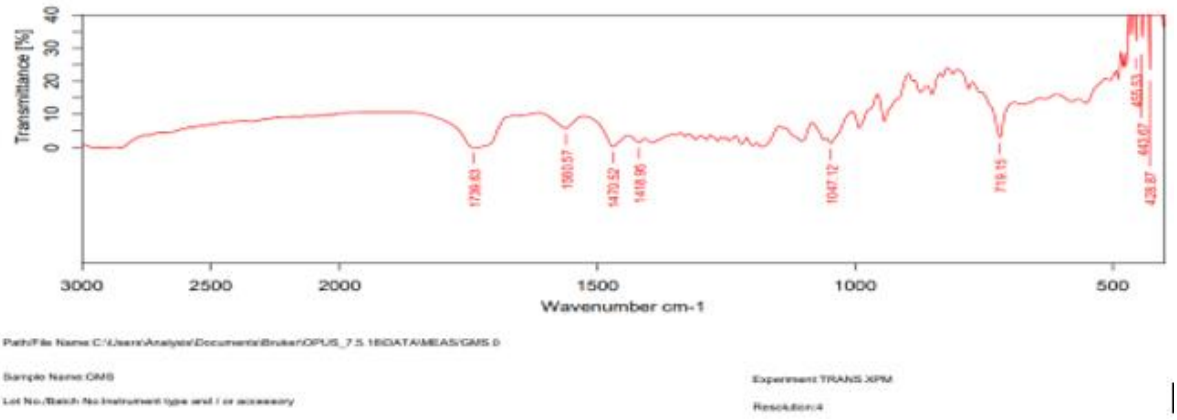

Figure 1.5: FTIR spectra of Glycerol Monostearate

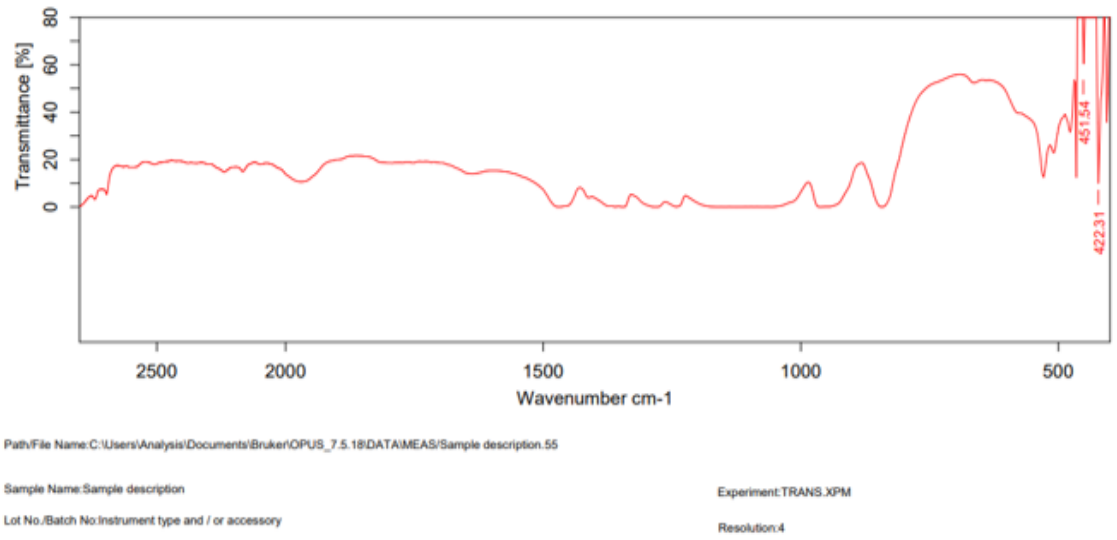

Figure 1.6: FTIR spectra of Tween 80

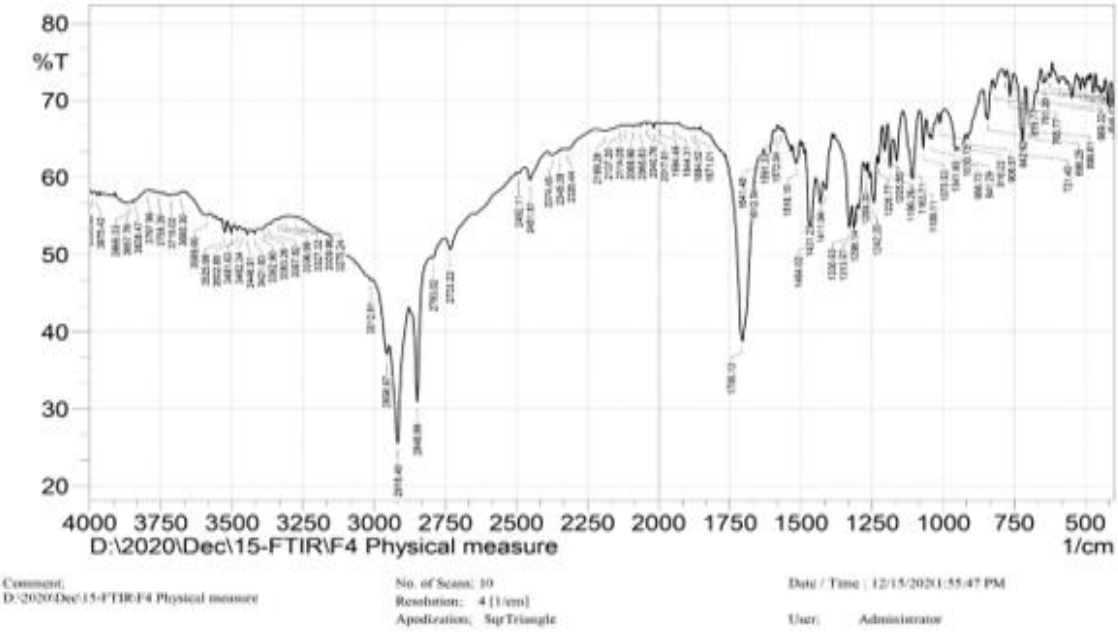

Figure 1.7: FTIR spectra of Physical mixture

The characteristic peaks of Fluoxetine, GMS appeared in physical mixture. This implies that the lipid is solidifying in SLN's into a matrix that is similar in structure to that of the original excipient.

4.5.2 DSC of Pure Fluoxetine

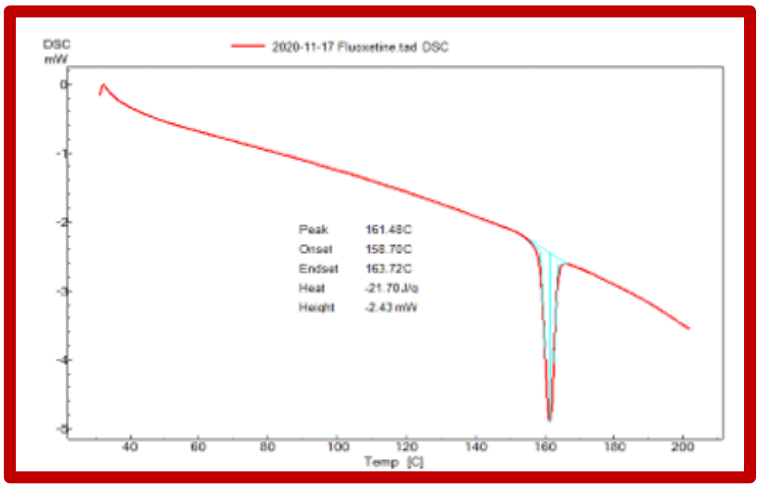

Figure 1.8: DSC of Fluoxetine Pure drug

The pure fluoxetine showed a sharp endothermic peak at 161.48 ℃ representing its melting point**.**

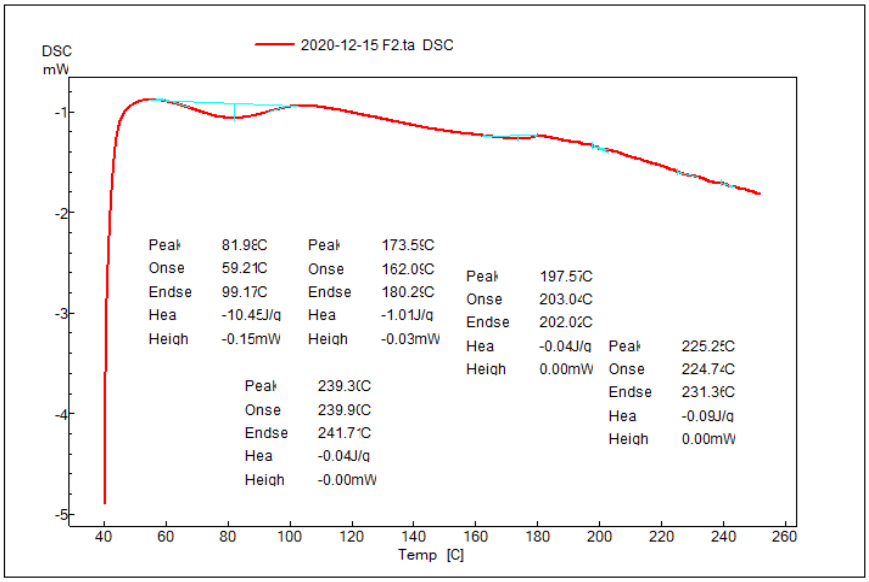

Figure 1.9: DSC of fluoxetine SLN formulation

DSC of SLN formulation showed a endothermic peak of drug at 173.54 ℃ & broad endothermic ranging from 81℃ to 239.3℃ representing changes in lipid crystallinity. This could also be attributed to solubilisation of drug in lipid phase to change in the molecular interaction in the similar lipid molecules.

4.5.3 Particle size, size distribution Zeta potential of OPT-*FLX-SLN*

The average particle size, PDI and Zeta potential of the solid lipid nanoparticles, was determined using Malvern Zetasizer Nanoseries Nano-ZS was found to be 108.5 nm, 0.172, -21.2 mV respectively as shown in Figure 1.10 & 1.11.

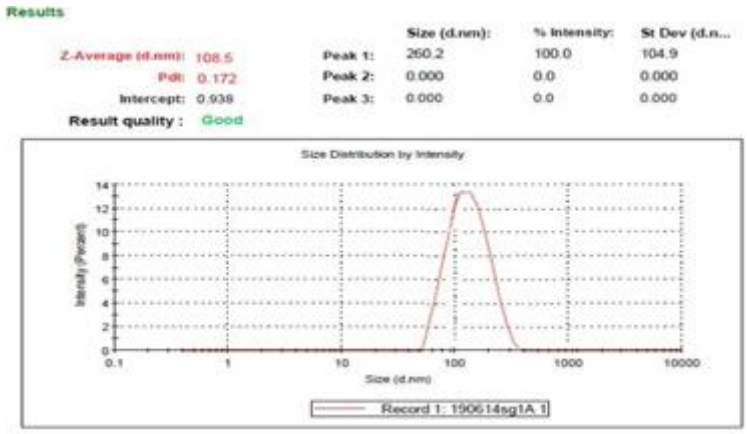

Figure 1.10: Particle size distribution of Optimized Formulation

Zeta Potential

Average particle size of less than 110 nm indicated the suitability of the formulation for administration through various routes with the potential of increased permeability and thus enhanced bioavailability of the drug Fluoxetine. Low PDI value (< 0.2) indicated the narrow distribution of size (monodispersity) and stability of the formulation was indicated by the zeta potential value (-21.2 mV).

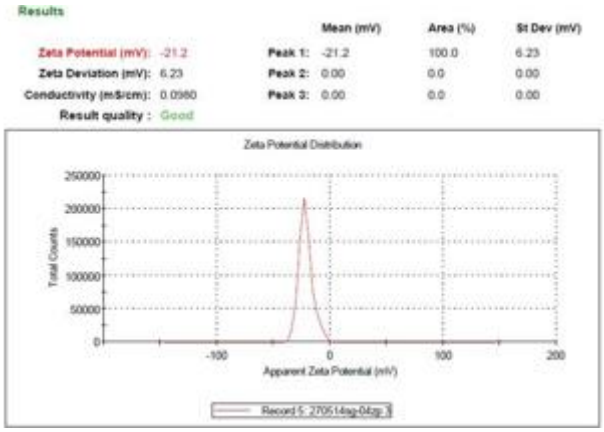

Figure 1.11: Zeta potential of Optimized Formulation

4.5.4 Scanning Electron microscope of Optimized Formulation

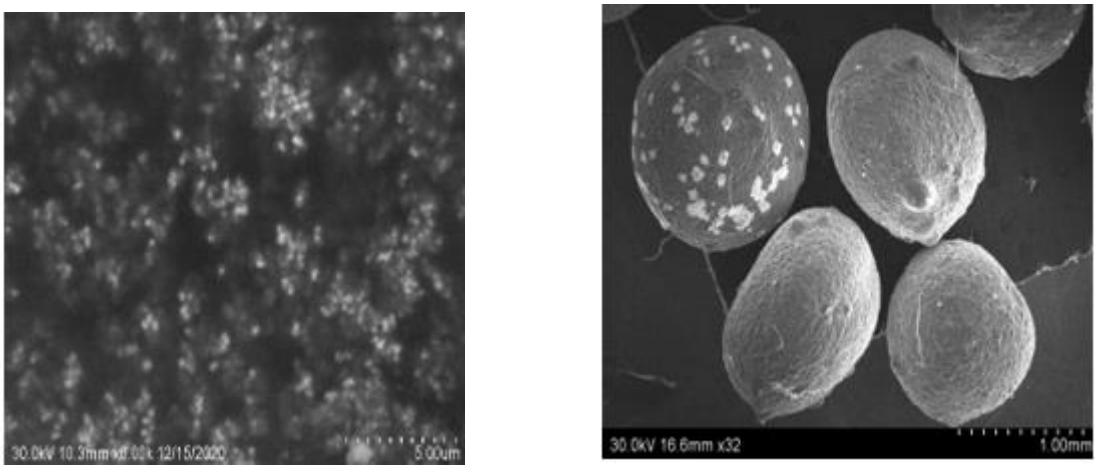

Figure 1.12: SEM images of Optimized Formulation

The surface morphology of the optimized SLN was investigated using Scanning electron microscope and the image was shown in Figure 1.12. Spherical particles were observed with drug incorporated in the lipid matrix.

4.6 Formulation of In-situ Gel

In order to increase the nasal residence time of the formulation, to achieve increased olfactory delivery to CNS and at the same time maintaining the ease of application of the formulation, in-situ gelling system incorporating the developed formulation was developed. Poloxamers are reported to have poor mucoadhesive properties. Hence, some mucoadhesive polymer is required to be added along with poloxamers to make effective in-situ intranasal gel formulation. Carbopol 934P was selected as gelling/ mucoadhesive agent.

Table 1.12: Formulation of drug loaded SLN in-situ gel

Ingredients	FLX-SLNG1	FLX-SLNG2	FLX-SLNG3	FLX-SLNG4	FLX-SLNG5
FLX-SLN-OPT	10	10	10	10	10
Carbopol 934 P	0.5 %	0.5 %	0.5 %	0.5 %	0.5 %
Poloxamer 407	18 %	22 %	24 %	26 %	28 %

4.7 Characterization of Fluoxetine loaded SLN gel

Table 1.13: Characterization of Fluoxetine loaded SLN-Gel

Batch code	pH ±S. D	Viscosity(cP)	Mucoadhesive strength (dynes/cm^2)	Drug content(%)
FLX-SLNG1	5.4±0.01	528±1.02	54.18±3.14	99.33±2.67
FLX-SLNG2	5.2±0.04	212±1.11	12.34±1.62	97.51±2.33
FLX-SLNG3	6.0±0.03	301±4.11	39.47±2.78	95.42±2.84
FLX-SLNG4	6.2±0.01	465±3.13	42.22±4.03	97.77±2.99
FLX-SLNG5	6.1±0.04	461±2.34	36.77±3.47	96.11±3.11

pH: The pH of the formulation was determined to be 5.4 which indicated non-irritancy of the formulation owing to the pH similar to that of nasal mucosal secretions. The pH of the nasal mucosal secretions ranges from 5.5 to 6.5 in adults and 5.0 to 6.7 in children.

Viscosity: Viscosity of the formulation is important since higher the viscosity of the formulation (after in-situ gelling on administration at nasal temperature), greater will be the contact time which increases the time for absorption.

Viscosity of the formulation was determined to be 528±1.02 cps at 34 ℃ (nasal temperature).

Mucoadhesive strength:

Mucoadhesive strength was determined by measuring the force required to detach the formulation from membrane, that is, detachment force. It was observed that Carbopol 934 P and Poloxamer 407 both had shown effect on mucoadhesive strength. As the concentration for

both polymers increases, mucoadhesive strength was also found to be increased. F1 had shown highest mucoadhesive strength, that is, 54.18±3.14

Drug Content:

The content of the Fluoxetine in the SLN was found to be from 99.33±2.67%.

4.8 *Ex vivo* permeation studies of Optimized Fluoxetine loaded SLN Gel

Table 1.14: *Ex vivo* permeation studies of FLX loaded SLN-Gel

Time (hr)	FLX-Plain Gel	FLX-Opt-SLNG1
0	0	0
1	1200	3131
2	1600	4008
4	1900	6277
8	3100	8010
12	5083	9100

The permeation profiles revealed that the drug permeation followed a biphasic pattern with rapid phase for first 2 hour followed by slow phase from 2 to 12 hr. The drug permeation was rapid for **FLX -Opt-SLNG1** (9100µg) and more when compared to the **FLX -Plain Gel** (5083 µg) and it was also increased up to 12hr

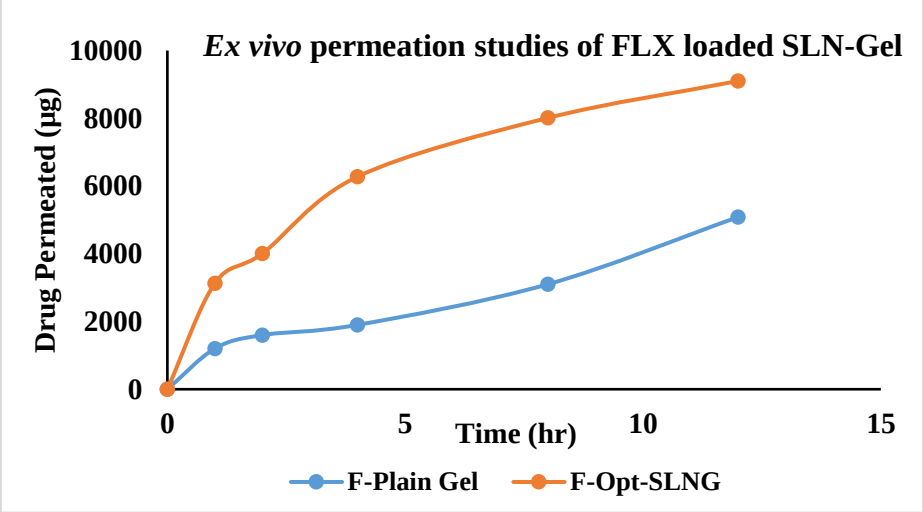

Figure 1.13: Comparison of Optimized gel with Plain gel

4.9 Stability Study of Optimized formulation of Fluoxetine loaded SLN- nasal gel

Table 1.15: Stability studies of Optimized formulation

Time (Months)	Drug Content%	Viscosity	Mucoadhesive Strength
Refrigeration temperature (i.e., 5 ± 2 °C)			
Initial	97.70	466	42.23
1	97.72	465	42.21
3	97.73	466	42.23
6	97.74	467	42.22

Room temperature (25 ± 2 °C/ 60 ± 5% RH)			
Initial	97.72	467	42.21
1	97.70	466	42.22
3	97.73	466	42.23
6	97.75	467	42.23

Stability study of optimized formulation was done at both Refrigeration temperature **(5 ± 2 °C)** and 25℃/60 ± 5 % RH for 6 months and result was depicted in Table 1.15**.** During and at the end of the accelerated stability study, the selected gel formulations showed drug content similar to what was observed at the beginning of the study. These formulations exhibited satisfactory mucoadhesive strength and viscosity during and at the end of the accelerated study period.

Conclusion:

In this research, Fluoxetine loaded Solid lipid nanoparticles (SLN's) gel was successfully developed, optimized using the systematic approach of design of experiments (DoE) and evaluated for their efficacy to the brain. The chosen experimental design for response surface methodology, i.e., Box-Behnken design, mathematical model for generation of optimized formulation Optimized formulation was prepared and found to be PS of 101.36 nm, EE 81.56 and Drug release at 24 hr 96.31% respectively. The permeation profiles revealed that the drug permeation followed a biphasic pattern with rapid phase for first 2 hour followed by slow phase from 2 to 12 hr. The drug permeation was rapid for **FLX -Opt-SLNG1** (9100µg) and more when compared to the **FLX -Plain Gel** (5083 µg) and it was also increased up to 12hr.

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