RESEARCH ARTICLE

Development and Evaluation of Nanoformulation of an Anti-Viral Drug for Brain Targeting

Bashyal B R[1*], Sarangdevot Y S[2], Chauhan C S[3], Vyas B[4]

[1]*Research Scholar, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan), india-313001*
[2]*Professor, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan), india-313001*
[3]*Professor, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan), india-313001*
[4]*Associate Professor, Faculty of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan), india-313001*

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ABSTRACT

HIV remains a devastating infection and a leading cause of mortality worldwide, ranking sixth globally. Unfortunately, non-nucleoside reverse transcriptase inhibitors (NNRTIs), a crucial class of HIV treatments, face significant challenges that mainly include first-pass metabolism, protein binding, and enzyme metabolism. The formulation of Nevirapine loaded SLN has improved absorption and brain targeting. Using a systematic set of experiments, that mainly processed by high-pressure homogenization for the production of solid lipid nanoparticles (SLNs) and analysed particle size, PDI, zeta potential, and entrapment efficiency. Scanning electron microscopy shows the morphological properties and revealed spherical and irregular lipid nanoparticles. The following parameters were evaluated for the Nevirapine SLN gel: gel temperature, pH, viscosity, light transmittance, muco-adhesive strength, diffusion, and *in vitro* and *ex vivo* dissolution experiments. Nanotechnology-based anti-viral medications offer promise in overcoming HIV treatment challenges, potentially eliminating viral reservoirs in the brain and curing patients through intranasal delivery, as evidenced by *in vivo* pharmacokinetic studies. Nanoparticle design parameters – composition, shape, size, and characteristics – can enhance: Anti-viral activity, Stability and Effectiveness of the formulation. This research work explores- types of antiviral medications and efficacy concerns, nanoparticle-based drug delivery systems, current research and future perspectives.

Keywords: Nanotechnology, HIV, Solid Lipid Nanoparticle, Nevirapine

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INTRODUCTION

In the recent scenario of drug delivery system, nano-technology has a crucial role in development of newer formulations for targeted, controlled, and sustained drug delivery system. Nano-technology has a great impact in the improvement of bio-availability of drug and the dosage form with plays an important role to overcome the quantity of drug required for the treatment of disease.[1] Nanotechnology helps to improve wide range of formulations for drug delivery system, drug targeting, to improve drug delivery techniques, enhance the efficacy of drug as well as to reduce toxicity and amount of drug required to produce effect.[2,3] Advantages of nanotechnology in drug delivery, especially for taking drugs by mouth, include the ability to deliver drugs that are not soluble in water, precisely target certain areas of the digestive system, transport drugs across the gut barrier, and deliver big molecules to cells or to the cells themselves. Nanotechnology has brought forth a variety of nanoparticles, including polymer nanoparticles, liposomes, nanoparticles with special properties, solid lipid nanoparticles (SLNs), micelle-like structures, quantum dots, dendrimers, tiny capsules cells, cell-like structures, lipoproteins, and various combinations of these nanoparticles.[4,5] Using nanotechnology in oral drug delivery can enhance the effectiveness, selectivity, safety, and potential benefits of the drugs being used. Here's a simplified breakdown of how nanotechnology in oral drug delivery can improve the effectiveness and safety of drugs.[6] Solid Lipid Nanoparticles (SLNs) are innovative nanocarriers designed to enhance the delivery of water-i n soluble medications and cosmetic active ingredients. These colloidal particles, ranging from 10 to 1000 nanometers in size, offer improved drug delivery and reduced toxicity. SLNs have emerged as a promising alternative to traditional carrier systems, providing improved performance and minimized side effects.[7] Ongoing research and development are exploring new applications and optimization strategies for these versatile nanocarriers.[8,9] The major applications of SLNs include- Cancer (targeted delivery), Infectious Diseases (antimicrobial delivery), Neurological Disorders (blood-brain barrier targeting), Dermatological Conditions (topical delivery), Ophthalmic Diseases (ocular delivery) .[10]

**Author for Correspondence: bashyal2002@gmail.com*

Table 1: Identification tests for Nevirapine with the inferences

Parameters	Observations	Reported	Inferences
Appearance	White powder	White to dusty white powder	Confirmed
Solubility	Poorly soluble in water, sparingly soluble in 0.1N HCl and methanol	Poorly soluble in water, sparingly soluble in 0.1N HCl and methanol	Confirmed
	Soluble in Dimethyl suphoxide, sparingly soluble in Dichloromethane and in Dimethyl formide.	Soluble in Dimethyl suphoxide, sparingly soluble in Dichloromethane and in Dimethyl formide.	Confirmed As per IP
Melting point	244-248^{0}C	245-247 ^{0}C	Confirmed

MATERIAL AND METHOD

Identification of Drug Sample

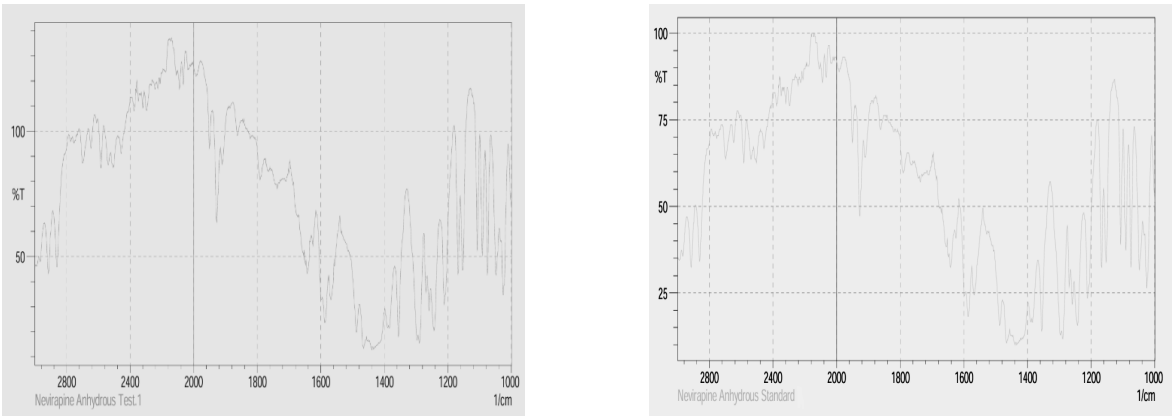
In order to ensure the quality and purity of procured drug sample, it becomes mandatory to perform the preliminary tests before the development of the formulation.[11] The major tests that were done for the identification of sample includes appearance of drug sample, solubility, melting point determination and confirmation was done by fourier-transform infrared (FT-IR) spectroscopy to detect various functional groups present in the powder drug sample.[12]

Solubility

The phase solubility of the drugs was determined in 0.1N HCl. The solubility of drugs nevirapine was determined by taking an excess amount of drugs (10 mg) individually, and added them in 10 ml of above solvent, in vials. The samples were kept at equilibrium for a period of 24 hrs in incubator at 37± 0.5ºC with occasional shaking.[13] The supernatant collected from vials was filtered through Whatman filter paper after filtration a sample for analysis can be taken and analyzed by UV-Visible spectrophotometer (Shimadzu) at respective wavelength. The shake flask method is the most accurate method to determine solubility but it is time consuming.[14,15]

Determination of Melting Point

The Melting point of nevirapine was individually determined using open capillary method. The capillaries loaded up with powder were placed in Melting point apparatus containing fluid paraffin. The capillary loaded up with drug powder was kept in Thiel's tube containing fluid



(a) (b)

Figure 1: IR spectra (a) Observed spectra of nevirapine (b) Reported spectra of nevirapine Standard

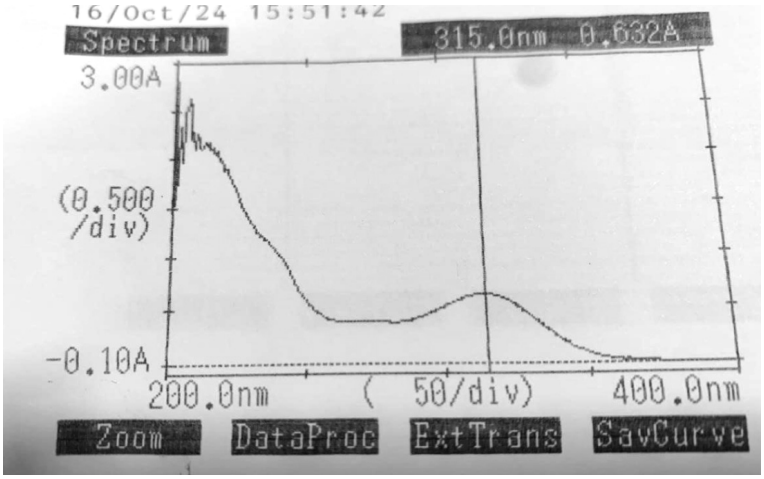
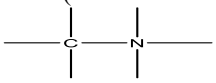


Figure 2: UV Spectrum of Nevirapine

Table 2: Important observed peaks of Nevirapine in IR spectra

Inferences	Observed(cm^{-1})
-C-C- (aromatic ring)	1586.64, 1568.95.
-OH	3295.51
=C-H (alkenes)	3188.96,3011.53,3124.65,3062.17
-C-H-(mono substitution)	884.74,829.67,803.64
-C-H-(Di substitution)	789.05, 761.85.
 (Substituted amines)	1025.27, 1047.92, 1074.70, 1090.97

paraffin. The tube was warmed and the liquefying point of the medication powder was noted (16).

In Fourier Transform Infrared (FT-IR)

The identification of the drug was performed using infrared spectroscopy with Shimadzu, IR Prestigue-21 FTIR spectrophotometer. The sample was prepared using the disc method. Approximately 5 mg of the drug was triturated with 100 mg of dry potassium bromide (KBr) in a mortar-pestle to create a fine, uniform mixture.

UV Spectroscopy

In order to estimate the nevirapine in formulations, the UV-Spectroscopy was done using UV-1800 spectrophotometer (Shimadzu), and calibration of sample was done using 0.1N HCl.

Calibration Curve of Nevirapine in 0.1N HCl as Solvent

For calibration of drug sample nevirapine, the solvent composition preferred 0.1N HCl for analysis.[15]

Preparation of Stock-I Solutions

An accurately weighed 100 mg quantity of nevirapine was transferred to a 100 ml volumetric flask. The nevirapine was dissolved and the volume was made up to the mark with 0.1N HCl to obtain a final concentration of 1000 µg/mL. 1 mL of the stock solution was further diluted to 10 mL with the same solvent (0.1N HCl) to obtain a working solution with a concentration of 100 µg/mL.

Preparation of Stock-II Solutions

Nevirapine stock solution (100 µg/mL) was used to prepare calibration standards. Calibration Standards Preparation: Volumes of stock solution (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 mL) were pipetted into 10 mL volumetric flasks and diluted to the mark with solvent (0.1N HCl). ranging from 5 -40µg/mL. Mean absorbance values (n=3) plotted graphically against concentration.[16]

Formulation and Optimization of In-situ Gel

The formulation of solid lipid nanoparticles was done using by mixture of the lipid phase as well as the aqueous phase. The drug Nevirapine was melted to produce lipidic phase whereas mixture of water and surfactant aims in the formulation of aqueous phase of the preparation. By the

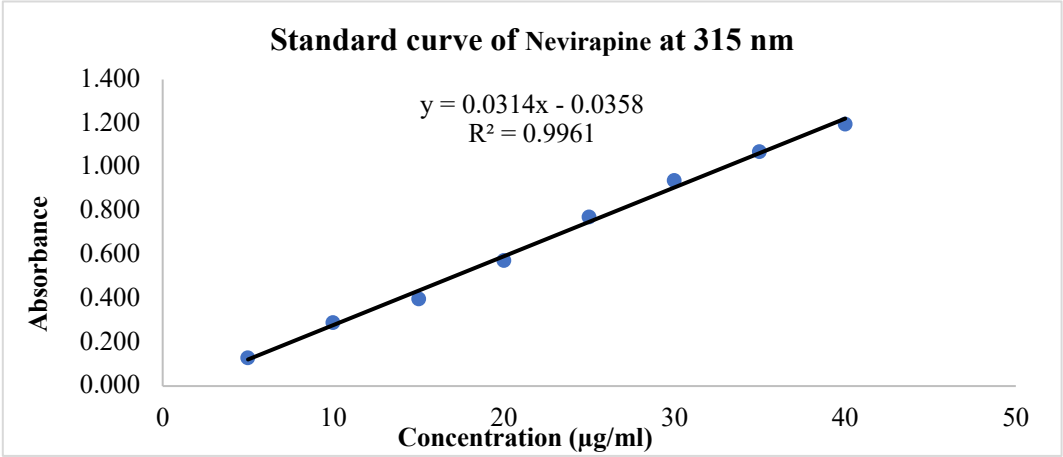


Figure 3: Standard curve of Nevirapine

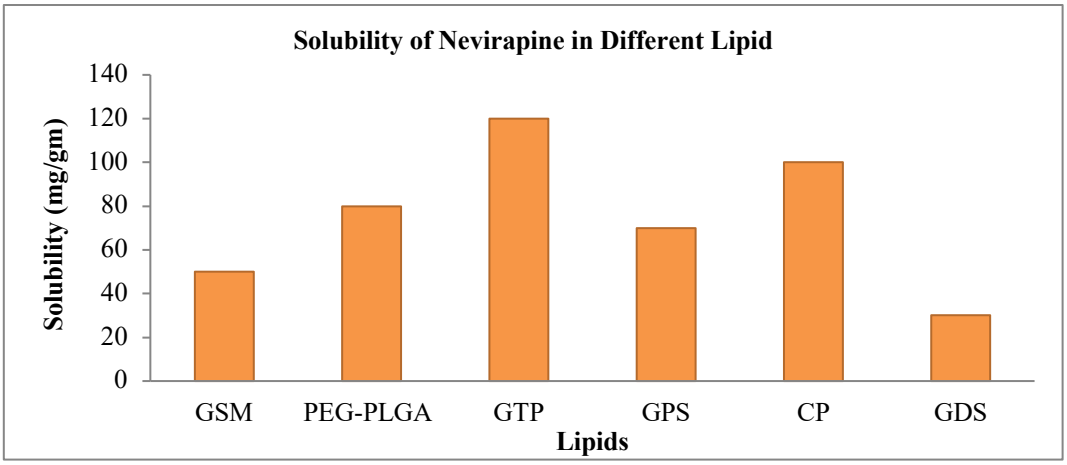


Figure 4: Solubility of Nevirapine in different lipids

process of continuous stirring, formulation of o/w emulsion takes place which upon homogenization with high pressure results in the formation of o/w nano emulsion. Cooling and sonication of o/w nano emulsion produces drug - Nevirapine loaded solid lipid nanoparticles.[17]

This Nevirapine loaded solid lipid nanoparticle was incorporated to produce in-situ-gel by using Carbopol 934P and chitosan as gelling / mucoadhesive agent.[18]

Optimization of Formulation Variables

The formulation variables were optimised using a 3^2-factorial design, and each response's contour plot and 3D surface plot were created using SAS Analytics Pro software, which was utilised for statistical analysis via ANOVA and model equation generation. Particle size, PDI, and entrapment efficiency were the key quality features chosen as answers, and the amount of drug in relation to lipid and surfactant concentration were examined as independent variables at three levels.[19]

Evaluation Parameter from Optimized Formulation

Solubility Studies of Nevirapine Solid Lipid Nanoparticles

The formulation with highest solubility between different formulations was selected as optimized formulation. According to the solubility analysis, the formulation containing the medication is estimated by Shake flask method.[20]

Table 3: Standard Graph of Nevirapine at 315nm in 0.1N HCl

Sr. No.	Concentration (µg/ml)	Absorbance 1	2	3	Average Absorbance
1	5	0.128	0.125	0.136	0.130
2	10	0.292	0.287	0.292	0.290
3	15	0.399	0.393	0.402	0.398
4	20	0.574	0.569	0.579	0.574
5	25	0.771	0.768	0.776	0.772
6	30	0.981	0.915	0.921	0.939
9	35	1.083	1.035	1.095	1.071
8	40	1.204	1.178	1.212	1.198

Correlation Co-efficient (R^2) = 0.9961

Absorbance (y) = 0.0314x conc - 0.0358

Based on Practical Yield

The formulation with highest practical yield between different formulations was selected as optimized formulation.[21]

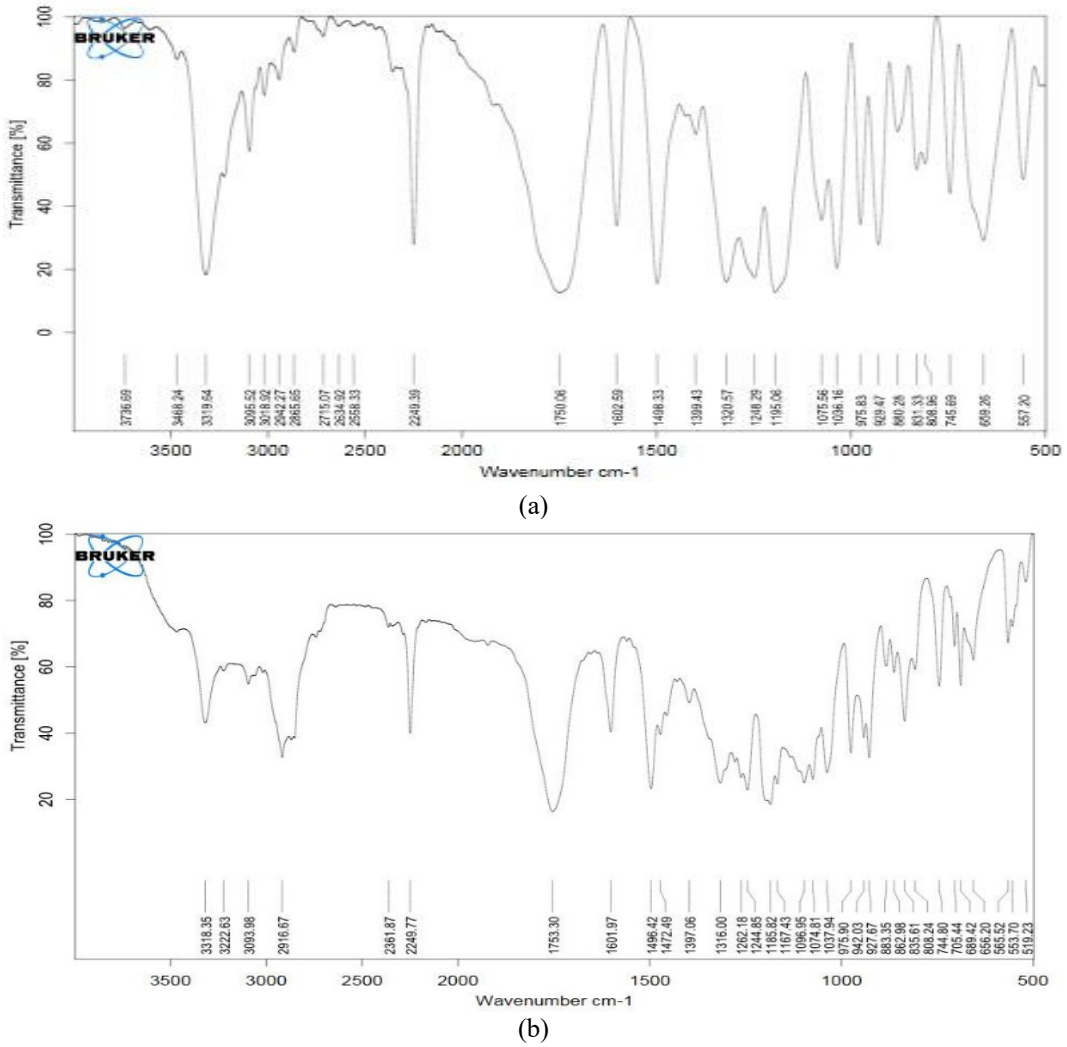


Figure 5: IR spectra of drug and physical mixture of drug and excipients. (a) IR spectrum of drug (Nevirapine) (b) IR spectrum of drug (Nevirapine) + Lipid (Tripalmitin) +Surfactant (Polysorbate 80)

Table 4: Selection of lipid based on solubility of drug in lipid

Lipids	Melting Point(^{0}C)	Solubility of drug in lipid (mg/g)
Glyceryl monostearate	58-59	40-60
PEG-PLGA Copolymer	50-60	70-90
Tripalmitin (Glyceryl tripalmitate)	66-68	110-130
Glyceryl palmitostearate	53-57	60-80
Glyceryl distearate	65-77	20 -40
Compritol 888 ATO (Glyceryl behenate)	70-71	40-60

***In-vitro* Dissolution Studies**

The formulation with highest Dissolution between different formulations were selected as optimized formulation. Both the Franz-diffusion cell and the dialysis- bag/dialysis-sac method were used to acquire the in-vitro drug diffusion profile of the marketed drug and nevirapine loaded SLN dispersion. Activated dialysis membrane bags (Dialysis membrane 110 (LA 395), Himedia, cut off 12000 Da) were filled with SLN dispersion and marketed product for the dialysis bag method.[22]

The bags were then suspended in glass beakers filled with 50 ml of methanolic phosphate buffer saline (pH 6.4, 40%v/v). To stop any evaporative loss throughout the experiment, the beakers were covered with paraffin film, set on magnetic stirrers, and swirled with magnetic beads. After aliquots were taken out of the receptor compartments at regular intervals till one hour and replaced with equivalent volumes of brand-new diffusion medium. Spectrophotometric analysis of the aliquots was performed at 283 nm.[23]

The activated dialysis membrane (Dialysis membrane 110 (LA 395), Himedia, cut off 12000 Da) was placed on a Franz diffusion cell with receptor compartments filled with methanolic phosphate buffer saline (pH 6.4, 40%v/v) or for in-vitro release experiments. Nevirapine formulations (0.5 ml) were kept in the donor compartments while the cells were set up on magnetic stirrers. Aliquots were taken out of the receptor compartments at regular intervals and replaced with equivalent volumes of brand-new diffusion medium. Spectrophotometric analysis of aliquots was performed at 283 nm.[23]

Evaluation of SLN Based In-situ Gel

Gelation temperature, gelation time, pH, viscosity, transmittance, mucoadhesive strength, and spreadability were all assessed for the prepared SLN-based gel.

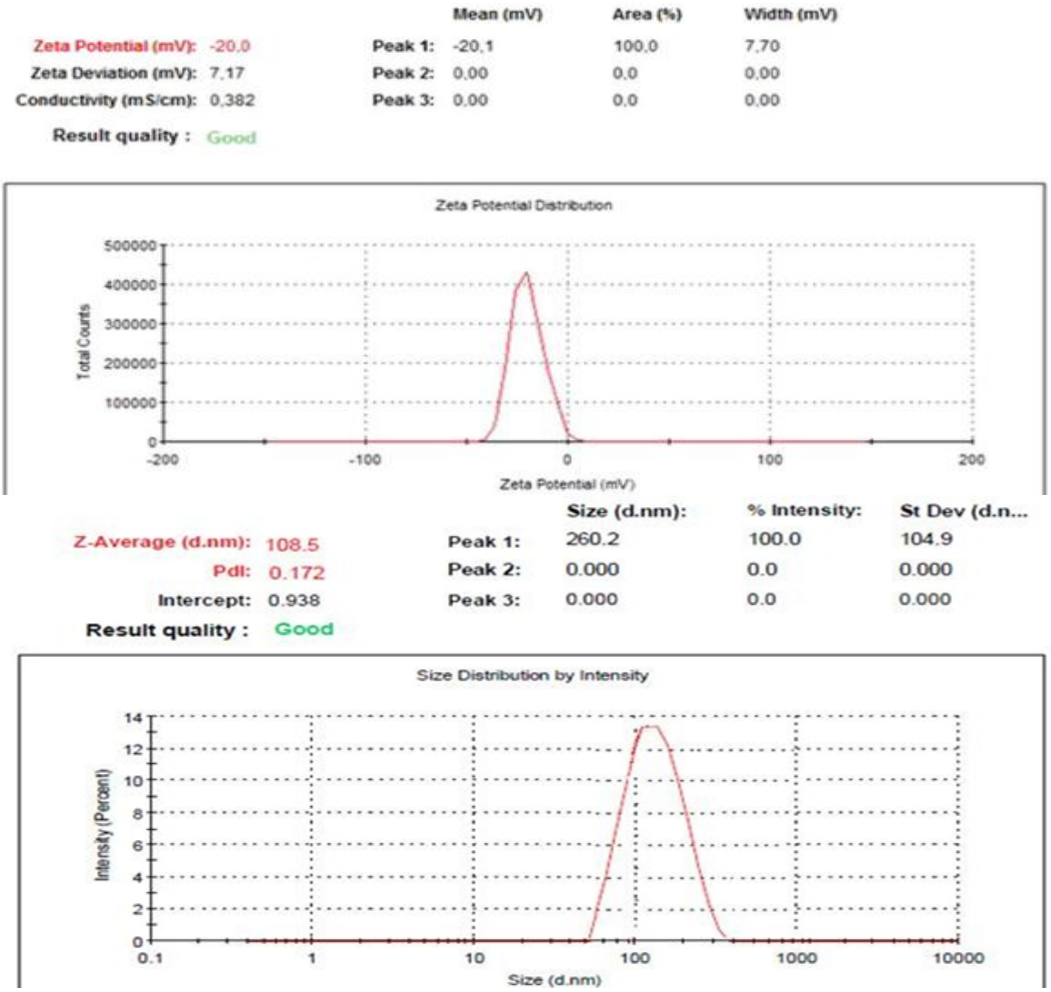


Figure 6: Size distribution and zeta potential distribution

Table 5: Selection of Surfactant on the basis of Particle size, PDI and Entrapment Efficiency

Sr. No.	Lipid	Surfactant (1% w/w)	Particle size*(nm)	PDI*	Entrapment Efficiency*(%)
1	Tripalmitin	Polysorbate 80	562.8±7.5	0.386±0.2	24.08±0.42
2	Tripalmitin	Polysorbate 60	881.1±6.1	0.398±0.5	17.08±0.47
3	Tripalmitin	Polysorbate 20	596.5±5.9	0.530±0.2	19.15±0.72
4	Tripalmitin	Poly ethylene oxide	602.3±7.3	0.468±0.4	17.22±0.49
5	Tripalmitin	Oxyethylene	618.3±5.4	0.498±0.3	20.18±0.70
6	Tripalmitin	Sucrose esters	624.9±6.9	0.500±0.4	19.85±0.69

{PDI: Polydispersity Index, *Data expressed as mean± SD (n=3), #indicates statistical significance ($p < 0.05$)}

Temperature of gelation / temperature of sol-gel

Table 6: Comparison of formulation techniques on the basis of Particle size, PDI and Entrapment Efficiency

S. No.	Formulation	Technique	Particle size*(nm)	PDI*	Entrapment Efficiency*(%)
1.	F01	High Pressure Homogenization	$356.3^{\#} \pm 7.3$	0.393 ± 0.109	$73.84^{\#} \pm 1.7$
2.	F02	Solvent Evaporation method	$476.3^{\#} \pm 7.8$	0.420 ± 0.101	$54.76^{\#} \pm 1.9$

{PDI: Polydispersity Index, *Data expressed as mean ± SD (n=3), # indicates statistical significance ($p<0.01$)}

Table 7: Ingredients of various formulation of Nevirapine solid Lipid nanoparticle

Sr. No.	Formulation	Nevirapine (mg)	Tripalmitin (mg)	Polysorbate 80 (mg)	Purified Water (ml)
1	SE1	100	1000	50	100
2	SE2	100	1000	100	100
3	SE3	100	1000	150	100
4	SE4	100	1000	200	100
5	SE5	100	1000	250	100
6	SE6	100	1000	300	100
7	SE7	100	1000	350	100
8	SE8	100	1000	400	100
9	SE9	100	1000	450	100
10	SE10	100	1000	500	100
11	SE11	100	1000	550	100
12	SE12	100	1000	600	100
13	SE13	100	1000	650	100
14	SE14	100	1000	700	100
15	SE15	100	1000	750	100
16	SE16	100	1000	800	100
17	SE17	100	1000	850	100
18	SE18	100	1000	900	100

transition (Tsol-gel): Sol-gel transitions are essential to determining the formulations' efficacy.[25]

A glass vial containing two millilitres of the sample was set up on a magnetic stirrer set to 15 revolutions per minute with heating. Ten degrees Celsius per minute was the rate at which the temperature was raised gradually.[26]

Gelation Time

In order to calculate the lag time, the amount of time needed for gelation was also examined. A glass vial containing two millilitres of the sample was placed on a magnetic stirrer set at the gelation temperature (15 rpm), and the amount of time needed for gelation was noted.[27]

pH

To prevent irritation of the nasal mucosa, the pH of the nasal formulations is also crucial. Therefore, a pH meter

Table 8: Solubility studies of solid lipid nanoparticles prepared

S. No.	Formulation code	Solubility(mg/ml)
Pure drug	Pure drug	0.10
1	SE1	0.49
2	SE2	0.52
3	SE3	1.08
4	SE4	0.61
5	SE5	0.85
6	SE6	1.23
7	SE7	1.10
8	SE8	1.15
9	SE9	1.03
10	SE10	0.55
11	SE11	0.68
12	SE12	1.08
13	SE13	0.72
14	SE14	1.11
15	SE15	1.15
16	SE16	0.63
17	SE17	0.94
18	SE18	1.09

(Spectralab India, Accu pH-3) was used to measure the formulation's pH, and the results were compared to the nasal cavity's reported pH.[28]

Viscosity

Figure 7: Optimized formulation of Nevirapine solid Lipid nanoparticle

Another criterion that needs to be assessed for in-situ gels is viscosity. The formulations should have a viscosity that allows the patient to administer them comfortably.

Using a Brookfield viscometer (DV-I Prime), the formulation's viscosity was measured at ambient

temperature (25 ºC) and nasal temperature (34 ºC) .[29]

Transmittance

After diluting the nevirapine nanoparticles loaded in-situ gel with distilled water ten times, the resulting dispersion was visually inspected for any signs of turbidity. A UV spectrophotometer (UV-1800, Shimadzu) was then used to assess its transmittance percentage at 280 nm using distilled water as the blank.[30]

Spreadability

By sandwiching 0.5 g of gel between two cellophane membranes and pressing 100g of weight on it for a minute, the gel's spreadability was assessed. A measurement was made of the diameter of the area where the gel spread.[31]

Histopathological Studies

Isolated goat nasal mucosa was used for histological investigations. The nasal mucosa of a freshly separated goat was divided into four sections. PBS pH 6.4 was used as a negative control on one-piece, isopropyl alcohol, a mucociliary toxicity agent, was used as a positive control, and the SLN dispersion on the other two.[32] Each sample was carefully cleaned with distilled water after a day, fixed, dehydrated, embedded in paraffin wax, and stained with haematoxylin and eosin. The microtome (model 0126, Yorco, India) was employed for microtoming, and DPX was utilised as the mounting medium. Using an inverted microscope (NIKION TS-100), histological analyses were conducted to determine whether the formulation caused damage or irritation.[33]

***Ex-vivo* Drug Release Profile**

Franz diffusion cells were employed to carry out ex-vivo release studies. A freshly excised segment of goat nasal mucosa, measuring 5 cm in length and sourced from a local abattoir, rinsed three times with phosphate buffer at pH 6.4. Extraneous tissues were carefully excised using a surgical blade to isolate the mucosa. The prepared nasal mucosa was then placed in a Franz diffusion cell with a permeation area of 3.14 cm^2. To ensure stabilization, the receiving medium was stirred with a magnetic stirrer for 30 minutes in phosphate buffer at pH 6.4. Following stabilization, the phosphate buffer was replaced with 18 ml of fresh phosphate buffer at pH 6.4, maintained at 34°C. Subsequently, a formulation equivalent to 5 mg of

Table 9: % Practical yield & Drug content for different formulations of Nevirapine solid lipid nanoparticles

S. No	Formulation	%Yield	% Drug content
1	SE1	94.5	92.76
2	SE2	95.8	91.57

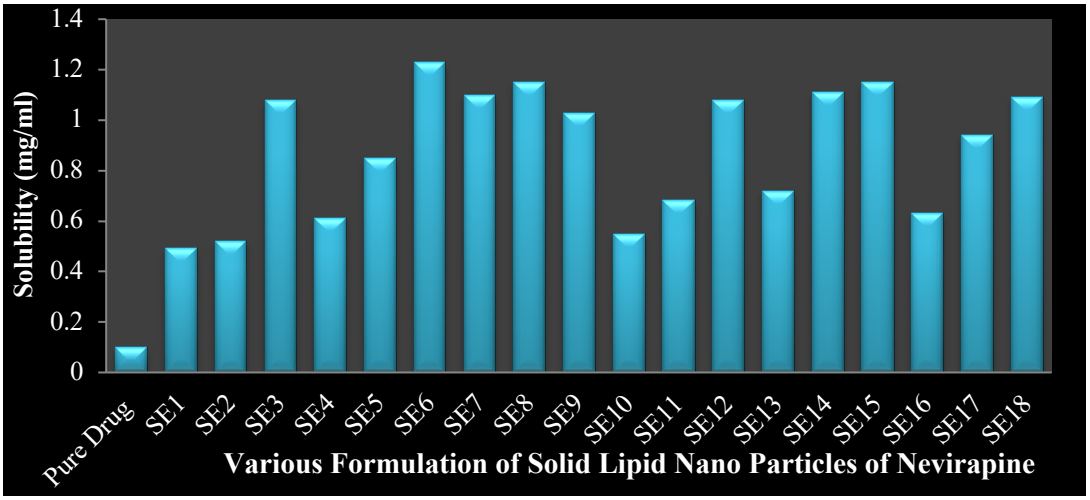


Figure 8: Solubility studies of solid lipid nanoparticles

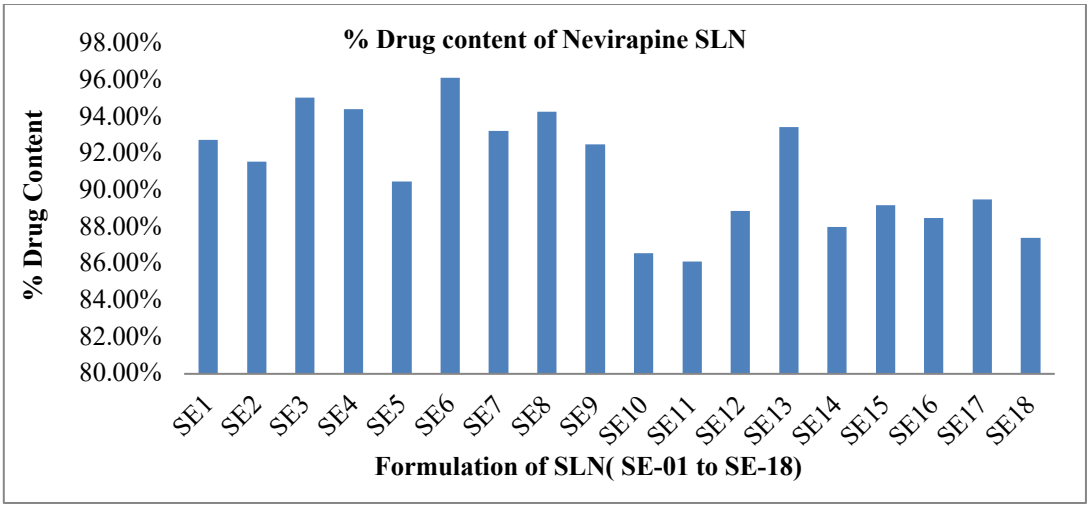


Figure 9: % Drug content of solid lipid nanoparticles

3	SE3	96.1	95.05
4	SE4	94.2	94.42
5	SE5	95.2	90.49
6	SE6	98.2	96.15
7	SE7	92.5	93.25
8	SE8	96.6	94.28
9	SE9	96.1	92.50
10	SE10	93.6	86.58
11	SE11	94.3	86.11
12	SE12	95.25	88.88
13	SE13	94.8	93.45
14	SE14	95.6	88.02
15	SE15	96.02	89.20
16	SE16	92.4	88.49
17	SE17	94.3	89.52
18	SE18	94.75	87.41

nevirapine was introduced into the donor chamber. At specified intervals (1,2,4,6,8,10,12,14,16,18,20,22 and 24 hrs), 1 ml samples were taken from the acceptor compartment and replaced with an equal volume of phosphate buffer at pH 6.4. The quantity of the permeated drug was quantified using a UV spectrophotometer at a wavelength of 283 nm for both the optimized formulation and marketed drug suspension.[34]

***In-vivo* Bioavailability Studies**

To determine the drug concentration, the brains were separated, weighed, and homogenised in PBS pH 6.4 at 5000 rpm using a Silent Crusher M homogeniser (Heidolph, Germany). The supernatant was then collected and centrifuged. The method developed and validated for measurement of nevirapine in plasma using HPLC was used to estimate the amount of drug in both the brain homogenate and plasma.

RESULT AND DISCUSSION

Drug Identification

The appearance of drug sample along with solubility and melting point determination was done with respect to estimation of the qualitative properties of the sample.

Fourier Transform Infrared Spectroscopy (FT-IR)

The sample's identity as a nevirapine medication is confirmed by its compliance with the stated requirements. FT-IR spectroscopy was used to demonstrate functional group and fingerprinting region of compound spectra. The observed result is then compared with the standard NVP spectra for verification. Figure 1 displays the reported and measured infrared spectra of NVP.

Spectrophotometric Determination of Nevirapine

Standard solution of Nevirapine was scanned in the UV range (200-400) and from the overlain spectrum; 315nm was selected as λ (lambda) max.

UV Spectroscopy of the drug sample- Nevirapine

Nevirapine scanned over a range of 400-200nm exhibited absorption maxima at 315 nm.

Calibration of Nevirapine

Standard curve for Nevirapine in 0.1 N HCl at 315 nm (Table 3)

Selection of Lipid

Glyceryl Monostearate, polyethylene glycol–poly lactic

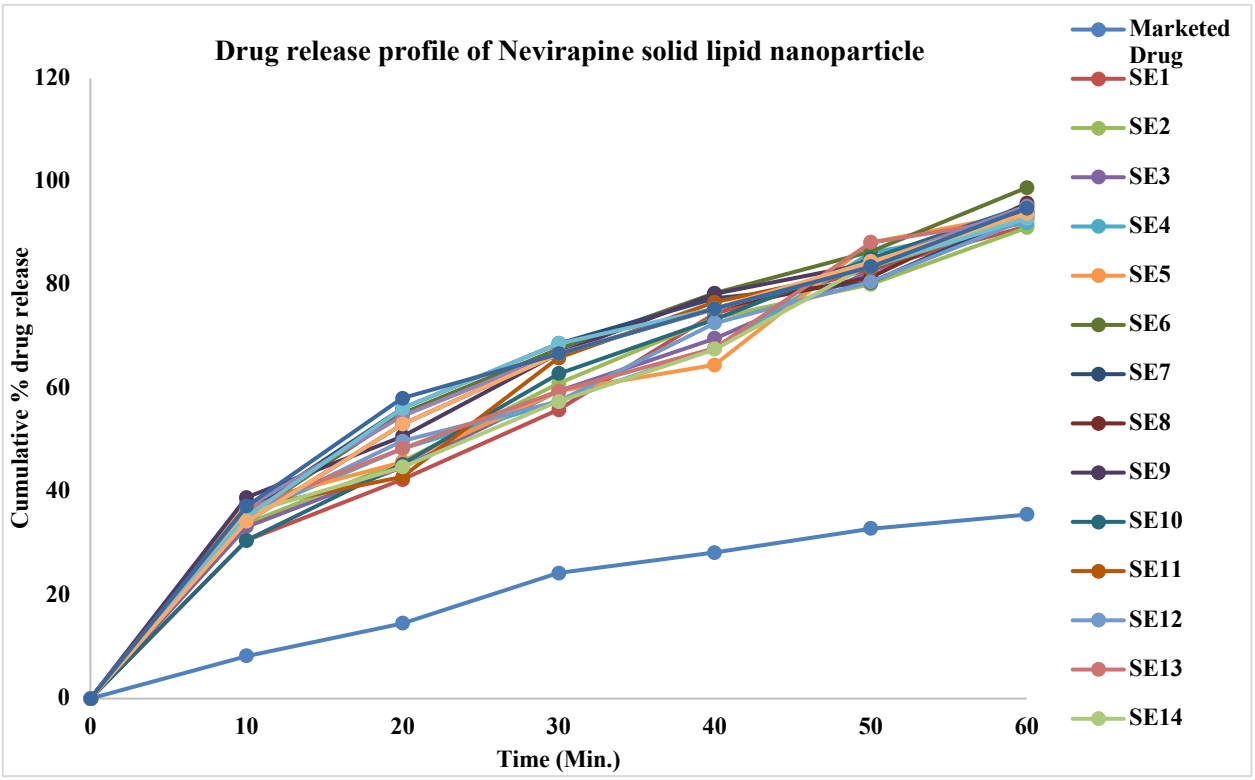
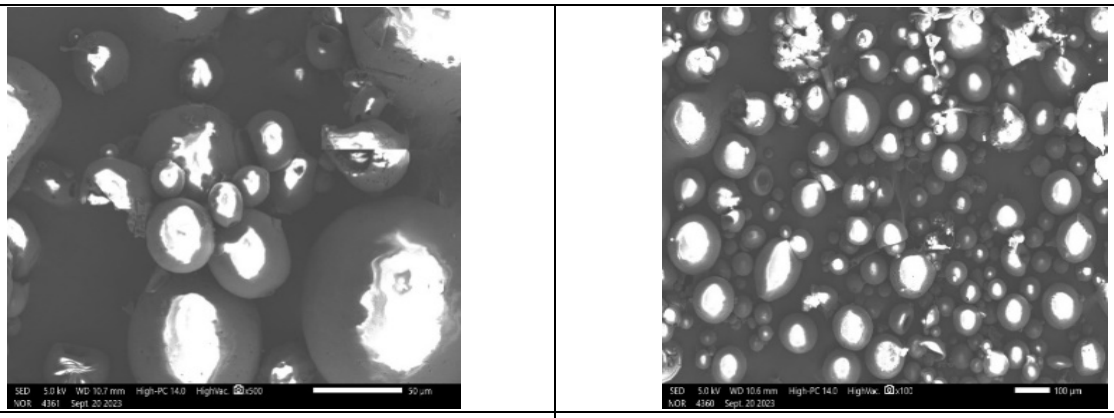
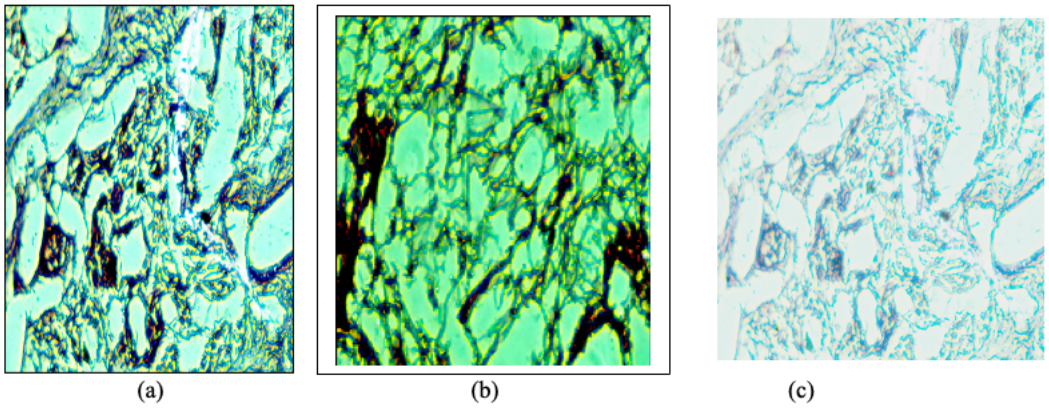


Figure 10: Cumulative % drug release of Nevirapine solid lipid nanoparticle (SE1-SE18) with Marketed product (Viramune tablets



(a) (b)

Figure 11: SEM photographs of Nevirapine pure drug (a) and optimized formulation SE 6 (b)



(a) (b) (c)

Figure 12: Histopathological conditions of nasal mucosa after treatment with (a) Phosphate buffer saline pH 6.4 (b) Isopropyl alcohol (c) SLN dispersion

acid-co-glycolic acid (PEG-PLGA), Glyceryl tripalmitate (Tripalmitin), Glyceryl palmitostearate, Glyceryl distearate and Glyceryl behenate (Compritol 888 ATO), were the six lipids in which the drug's solubility was assessed. The outcomes are displayed in Figure 4.

Polyethylene glycol–poly lactic acid-co-glycolic acid (PEG-PLGA) demonstrated the second greatest solubilising capacity of 80 ± 10 mg drug /gramme of lipid,

Table 10: Invitro Drug Release Profile of Nevirapine solid lipid nanoparticle SE1-SE18

Time (Min.)	0	10	20	30	40	50	60
Marketed Drug	0±0	8.23±0.21	14.55±1.44	24.31±1.51	28.22±0.05	32.89±1.11	35.61±0.78
SE1	0±0	30.58±0.45	42.37±1.65	55.89±1.28	74.44±1.22	83.20±1.34	91.38±2.15
SE2	0±0	33.86±2.44	45.86±0.62	60.94±0.75	73.68±1.65	80.14±1.75	91.14±1.55
SE3	0±0	33.29±0.68	44.80±0.89	59.50±0.86	69.69±0.59	82.69±0.85	93.81±1.86
SE4	0±0	35.60±0.98	48.40±1.55	57.50±1.75	67.76±1.78	86.27±0.18	92.08±0.58
SE5	0±0	37.86±0.21	45.81±1.44	59.49±1.51	64.57±0.05	88.21±1.11	94.04±0.78
SE6	0±0	35.23±1.45	55.12±2.26	67.72±0.18	78.45±1.19	86.56±0.52	98.89±2.32
SE7	0±0	36.23±1.45	56.12±2.26	68.72±0.18	77.45±1.19	80.56±0.52	93.89±2.32
SE8	0±0	34.23±1.45	53.12±2.26	66.72±0.18	75.45±1.19	81.56±0.52	95.89±2.32
SE9	0±0	38.93±2.15	50.72±0.98	66.77±1.42	78.36±1.59	84.23±0.78	94.31±0.11
SE10	0±0	30.58±0.44	45.37±1.64	62.89±1.27	73.44±1.21	85.20±1.33	95.38±2.14
SE11	0±0	36.86±2.45	42.86±0.63	65.94±0.76	76.68±1.66	83.14±1.76	93.14±1.56
SE12	0±0	35.29±0.77	49.80±0.85	57.50±0.82	72.69±0.53	80.69±0.80	92.81±1.82
SE13	0±0	36.60±0.92	48.40±1.51	59.50±1.79	67.76±1.73	88.27±0.11	93.08±0.55
SE14	0±0	35.86±0.29	44.81±1.46	57.49±1.54	67.57±0.15	84.21±1.17	93.04±0.70
SE15	0±0	36.93±2.19	54.72±0.90	66.77±1.43	75.36±1.56	84.23±0.74	95.31±0.16
SE16	0±0	35.23±1.43	56.12±2.23	68.72±0.16	75.45±1.11	83.56±0.57	92.89±2.36
SE17	0±0	34.23±1.45	53.12±2.26	66.72±0.18	75.45±1.19	84.56±0.52	93.89±2.32
SE18	0±0	37.23±1.40	58.12±2.29	66.72±0.13	75.45±1.17	83.56±0.57	94.89±2.34

Table 11: Different trials for selection of gelling/mucoadhesive agent for gel preparation

Sr. No.	Trials	Observations
1	Chitosan 100 mg +water 10ml - stirred with magnetic stirrer	Insoluble
2	Chitosan 100 mg +water 10ml - heat - stirred with magnetic stirrer	No proper gel
3	Chitosan 100 mg + water10ml (40^0C) -stirred with magnetic stirrer	Hydrated, lump formation
4	Acidic aqueous solution of chitosan+ Buffer pH 6.4	Soluble /Slight gelling
5	Chitosan 100 mg +10ml 0.1 M HCl- stirred with magnetic stirrer	Soluble/gelling
6	Chitosan 50 mg +5 ml SLN dispersion - stirred with magnetic stirrer	No proper gel
7	(Chitosan 100 mg +water 5 ml) + (polysorbate 80 150 mg + 5 ml water) heat - stirred with magnetic stirrer	No proper gel
8	(Chitosan 100 mg +water 5 ml) + (150mg polysorbate 80 + water 5 ml)- stirred with magnetic stirrer	Insoluble
9	Chitosan 100 mg + 6.4 pH buffer- heat - stirred with magnetic stirrer	Insoluble
10	Chitosan 100 mg + 6.4 pH buffer-stirred with magnetic stirrer	Insoluble
11	Carbopol934P + 10 ml water-stirred with magnetic stirrer	Gel formation
12	Carbopol 934P + SLN dispersion- stirred with magnetic stirrer	Gel formation

whereas glyceryl tripalmitate (tripalmitin) demonstrated the largest solubilising capacity of 120 ± 10 mg drug / gramme of lipid. Therefore, it was proven that the drug solubility in glyceryl tripalmitate was much higher than that of other lipids ($p < 0.05$). Compared to short chain. Tripalmitin was chosen for more research since it likewise has GRAS status 186.1555. SLN production using glyceryl tripalmitate has also been documented in a number of experiments.

Selection of Surfactant

Using tripalmitin as a lipid, nanoparticles were made with six distinct surfactants for surfactant selection, and their particle size, PDI, and entrapment efficiency were assessed and outcomes are displayed in Table 5.

Polysorbate 80 provided the PDI with the highest entrapment effectiveness and the smallest particle size. While the differences in PDI and entrapment efficiency were statistically non-significant, the difference in particle size was determined to be statistically significant when compared to others ($p < 0.05$).

An oily liquid derived from ethoxylated sorbitan (a derivative of sorbitol) esterified with fatty acids to form Polysorbate, Polysorbate are also said to sterically stabilise the particles and decrease the adsorption plasma proteins or opsonins on the surface. This can stop drugs containing SLN from being removed from the bloodstream. Bulky and non-ionic surfactants are generally thought to be less harmful than ionic and single-chain surfactants. Polysorbate 80, which also has food grade certification, was therefore chosen for research in the formulation.

Drug-excipient Compatibility Study

IR spectra of pure drug nevirapine and the physical mixtures of nevirapine, tripalmitin and Polysorbate 80 are shown in Figure 5.

As can be seen from Figure 5, the drug's principal peaks were all present in the IR, and the drug-excipients mixtures IR spectra showed no discernible changes, suggesting that the drug and the chosen excipients were compatible.

Selection of Formulation Technique

There are several methods for SLN formulation in which solvent emulsification and High-Pressure Homogenization was used in this study, out of which one method had been adopted based on Particle size, PDI, and entrapment efficiency were compared across SLN formulations made using various methods. The outcomes are displayed in Table 6.

It was found that high pressure homogenisation produced particles with a smaller size and a higher entrapment efficiency. While the difference in PDI was statistically negligible, the differences in particle size and entrapment effectiveness between the two methods were found to be statistically significant ($p<0.01$). Since organic solvent is not used in the high-pressure homogenisation process, there is no possibility of organic solvent residue. One significant toxicological drawback of the solvent evaporation process is the use of organic solvents. The high-pressure homogenisation technique's ability to be quickly scaled up for laboratory, pilot, or large-scale production is another benefit.

Particle Size, Polydispersity Index (PDI), Zeta Potential and Entrapment Efficiency

According to table 6, the solid lipid nanoparticles' average particle size, PDI, zeta potential, and entrapment efficiency, as measured by the Malvern Zeta sizer nano series Nano-ZS, were 356.3 nm, 0.393, -20.0 mV, and 73.84%, respectively. The majority of SLN created by high pressure homogenisation (HPH) have minimal microparticle content and an average particle size of less than 500 nm. A low PDI value (< 0.2) indicated the narrow distribution of size (mono-dispersity), and the zeta potential value (-20.1 mV) indicated the stability of the formulation.

The formulation's suitability for administration through various routes was indicated by an average particle size of less than 110 nm, which also had the potential to increase permeability and thus enhance bioavailability of the poorly soluble drug nevirapine. Hence High-Pressure Homogenization method had been chosen.

Preparation of Nevirapine Solid Lipid Nanoparticle

Total 18 formulations of Nevirapine SLN prepared from various carriers in varying compositions . All the formulations were fine, free flowing powders (figure 7), process of high-pressure homogenization technique. Formulation of in-situ gel was done after successful characterization and optimization of solid lipid nanoparticle dispersion. The developed in-situ gel loaded

with solid lipid nanoparticle of Nevirapine showed better absorption potential upto 14 times more effective with intranasal administration and 3.01 times more absorption upon oral administration of drug as compared to marketed oral dosage form availability. This proves that the optimized formulation of Nevirapine loaded in-situ gel provides better brain targeting that reduces the frequency of dose administration as well as the quantity of drug required to produce the desired actions. So the developed formulation upon necessary clinical trial investigations believes a great potential for the management of HIVs. From the observation, the drug sample was found to be Nevirapine.

Evaluation Parameter from Optimized Formulation

Solubility Studies of Nevirapine Solid Lipid Nanoparticles

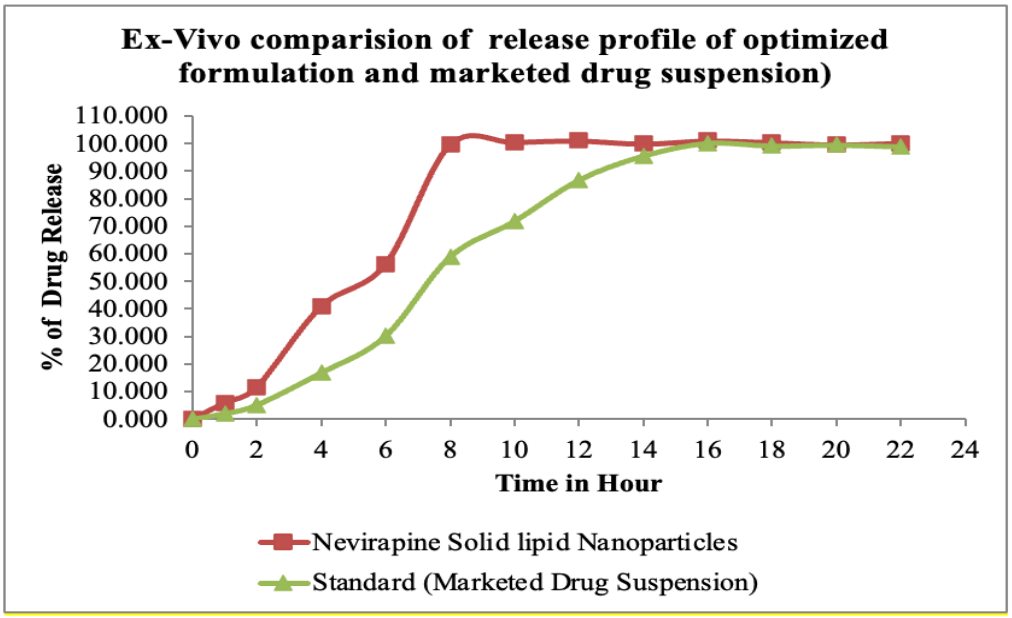


Figure 13: Graph of drug release profiles of Nevirapine in nasal mucosa of reference (marketed drug Dispersion) and Nevirapine-containing optimized solid lipid nanoparticles

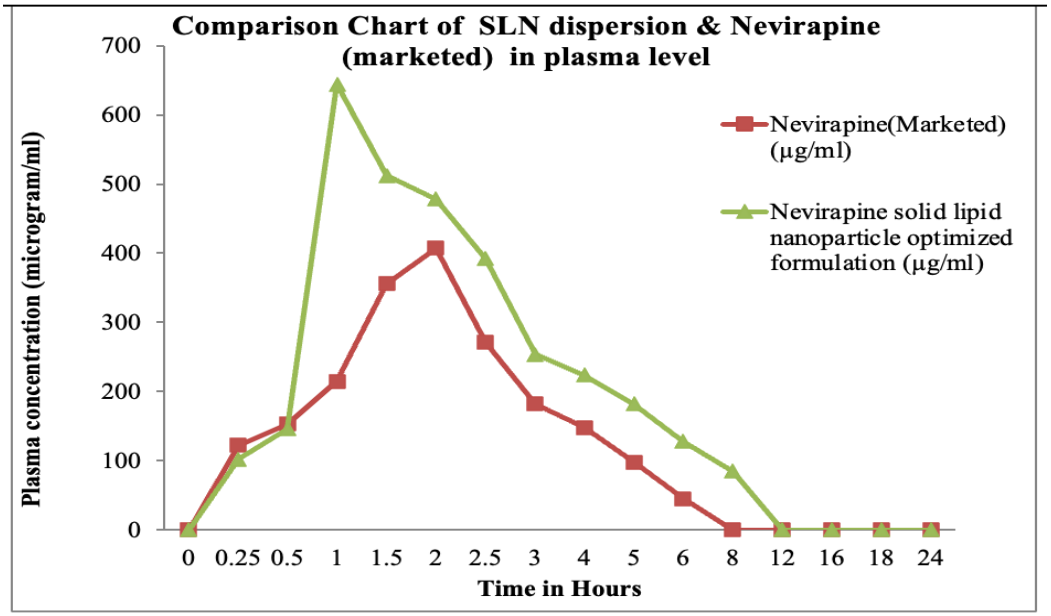


Figure 14: Graph of plasma profiles of Nevirapine in rats following oral administration of the reference marketed drug Dispersion) and Nevirapine-containing optimized solid lipid nanoparticles intranasally in brain

According to the solubility analysis, the formulation containing the medication Nevirapine: Polysorbate 80: Water (1:3:1) had the highest solubility, which was 12.3 times that of the pure drug (the solubility of the pure drug is 0.10 and that of the drug with carrier SE6 is 1.23 mg/ml). (Figure 8, Table 8).

Based on Practical Yield

The % Practical yield ranged within 92.4%-98.2% with maximum of 98.2% for SE6.The drug content ranged within 86.11% to 96.15% with maximum of 96.15% detailed for formulation SE6

***In-vitro* Dissolution Studies**

When compared to the release from marketed medication (35% in 60 minutes), the drug dissolution of SE1-SE18 showed an increase in the release rate of Nevirapine (Table 10).

According to the findings, formulation Nevirapine SE6, which contains a 1:3 ratio, showed higher release rates, or 98.89% in 60 minutes. Increased drug wettability, the presence of the drug in an amorphous state, and improved drug solubility by carrier are the causes of this increase in dissolution rate. (Fig. 10).

Table 12: Ingredients of various formulation of Nevirapine solid Lipid nanoparticle into Gel of optimized formulation SE6

Sr. No.	Formulation Nevirapine SLN	Carbopol 934 P(mg)	%w/v
1	SE6-100mg	100	0.1%w/v
2	SE6-100 mg	200	0.2 %w/v
3	SE6-100mg	300	0.3%w/v
4	SE6-100mg	400	0.4%w/v
5	SE6-100mg	500	0.5%w/v
6	SE6-100mg	600	0.6%w/v
7	SE6-100mg	700	0.7%w/v
8	SE6-100 mg	800	0.8%w/v
9	SE6-100mg	900	0.9%w/v
10	SE6-100mg	1000	1%w/v

Formulation and Optimization of In-situ Gel

Mucoadhesive nanoparticles may enhance olfactory delivery. Polysorbate 80 and Polysorbate 60 were chosen as surfactant. It has been found that Polysorbate has weak mucoadhesive qualities. To create an efficient in-situ intranasal gel formulation, Polysorbate must be combined with a mucoadhesive polymer. A variety of agents, including as carbopol 934P, chitosan, and others, are employed for mucoadhesive qualities and gelling.

Several mucoadhesive and gelling agents were evaluated in the current studies according to how well they gelled with SLN dispersion. Table 11 displays a variety of trials that were carried out. Carbopol 934P was chosen as the gelling/mucoadhesive ingredient because chitosan, even after heating, was unable to produce the correct gel formulation at a nasal pH of 6.4. According to the IR spectra of the pure medicine nevirapine and the physical combinations of nevirapine, tripalmitin, Polysorbate 80, and carbopol 934P, there was no discernible incompatibility between the drug and the chosen excipients.

Evaluation of SLN based In-situ Gel

Gelation Temperature/Sol–gel Transition Temperature (Tsol-gel)

When two millilitres of the sample were placed in a glass vial and heated on a magnetic stirrer, the gelation temperature was observed to be 34°C. The typical range of the nasal mucosal temperature is 30.2±1.7°C to 34.4±1.1°C. The gelation temperature of the majority of thermo-reversible gels that have been made and documented thus far ranges from 32 to 34°C.

Gelation Time

Less than a minute was found to be needed for full gelation at 34°C. The gelation at nasal mucosal temperature in less than a minute guarantees that the formulation won't flow when it's administered and lengthens the residence period. The formulation's pH was found to be 6.0, indicating that it was not irritating because it was comparable to the pH of nasal mucosal secretions. Adults' nasal mucosal secretions have a pH between 5.5 and 6.5, whereas children's range is between 5.0 and 6.7.

Viscosity

The formulation's viscosity was found to be 19,000 cps at ambient temperature (25°C) and 24,500 cps at nasal temperature (34°C).

Transmittance

Visual clarity and the absence of turbidity were seen in the NVP nanoparticles loaded in-situ gel diluted with distilled water (10 times dilution). At 280 nm , the transmittance percentage was 92% using distilled water as a blank.

Mucoadhesive Strength

The modified two-pan balancing method was used to calculate the mucoadhesive force. To separate the cellophane membrane from the gel, 18.5 millilitres of water had to be added. Thus, using this equation, the mucoadhesive force was determined to be 18150 dynes/cm². F= w x g=18.5 x981 dynes/cm² = 18148.5 dynes/cm² Where F is the mucoadhesion force (dynes/ cm²), W is the minimum weight required to break the bond (grams), g is the acceleration due to gravity (cm/s²).

Following intra-nasal delivery, proper hydrogel distribution on the nasal surface will guarantee improved drug absorption.

Scanning Electron Microscopy

Scanning electron microscopy was used to examine the optimised SLN's surface morphology. Figure 11 displays the image captured by a scanning electron microscope equipped with a CCD camera (TEM Philips Tecnai 20, Holland). Drug incorporated spherical particles were seen in the lipid matrix.

In contrast to Nevirapine SLN formulation (Figure 11b), SEM monograms show smooth crystals of pure medication (Figure 11a). The drug surface in the SD formulation is porous, giving it a homogeneous, wrinkled appearance. This demonstrates that the drug crystals seemed to be integrated into the polymer particles. The solid lipid nanoparticle had a matrix-like appearance. Drug dispersion in the polymer's molten mass may be the cause of the outcomes.

Histopathological Studies

The histopathological status of the nasal mucosa following treatment with PBS at pH 6.4 (negative control), isopropyl alcohol (positive control), and the formulated treatments is illustrated in Figure 12.

The safety of SLN formulations for nasal use was evidenced by the absence of significant damage or negative effects on the microscopic structure of the nasal mucosa treated with the SLN formulation, in contrast to the sample treated with isopropyl alcohol. Exposure to isopropyl alcohol resulted in a marked reduction of epithelial cells in the mucosa.

***Ex-vivo* Drug Release Profile**

The release profile of the drug from the solid lipid nanoparticle (SLN) dispersion demonstrated greater consistency compared to that from a marketed drug Dispersion. The SLN formulations exhibited an initial burst

release, followed by a sustained release pattern, aligning with findings from other studies. Over a 24-hour period, more than 90 % (99.582 %) of the drug was released from the SLN formulations, with a significant portion released within the first 8 hours. In contrast, the marketed drug dispersion showed less than 60% (58.925%) release over the same duration. The enhanced drug release can be attributed to the small size of the nanoparticles and the inclusion of

Table 13: Ex-vivo drug release profile of solid lipid nanoparticles and marketed drug dispersion

Time in hr	% of Drug release from Solid lipid Nanoparticles	% of Drug release from marketed drug Dispersion
0	0.000±0.48	0.000±1.25
1	5.955±0.68	2.020±1.51
2	11.567±1.11	5.120±0.78
4	40.913±1.81	16.820±0.95
6	56.313±0.94	30.150±1.11
8	99.582±0.97	58.925±1.36
10	100.520±1.56	71.896±1.62
12	101.057±1.80	86.852±1.91
14	99.940±0.18	95.600±0.81
16	101.067±0.24	100.250±0.76
18	100.453±1.68	99.250±1.19
20	99.610±1.79	99.590±1.08
22	100.240±1.52	98.980±0.79
24	100.45±0.99	100.025±1.19

Table 14: Pharmacokinetic Parameters of Nevirapine Optimized Solid lipid nanoparticle formulation, Nevirapine (marketed) in rat

Pharmacokinetic Parameters	Nevirapine solid lipid nanoparticle optimized formulation	Nevirapine marketed drug
Kel(h^{-1})	0.149±1.42	0.175±1.22
$t_{1/2}$(h)	4.65±0.04	3.96±0.01
T_{max} (h)	1.02±0.04	2.10±0.05
$AUC_{0\text{-}inf}$ (µg h/ml)	9758±0.45	4025±0.24
$AUC_{0\text{-}t}$ (µg h/ml)	7648±1.74	2620±1.55
C_{max} (µg/ml)	634±0.56	417±1.32

surfactants in the formulation, which likely improved permeability, wetting, solubilisation, and dissolution. The formation of pores in the matrix due to soluble surfactants may also contribute to the consistent and improved drug release from these nanoparticulate systems. Similar findings have been reported, indicating that nano-sized drug delivery systems can facilitate more effective nose-to-brain drug delivery compared to traditional drug solution formulations. This enhanced delivery may be partly due to the nanoparticles' ability to protect the drug from degradation and prevent its efflux back into the nasal cavity.

***In-vivo* Bioavailability Studies**

Table 14 and Figure 14 display the plasma pharmacokinetic parameters of Nevirapine following oral administration of the formulation to Wister rats. The findings confirm that, in comparison to marketed drug dispersion the new optimized formulation showed higher plasma concentrations.

The Cmax of the marketed drug dispersion, which was 407±1.32µg/ml, was much lower than the Cmax of the optimised solid lipid nanoparticle formulation, which was 644±0.56µg/ml. The Tmax for the marketed drug dispersion and the optimised solid lipid nanoparticle formulation was 2.00±0.05 hours and 1.00±0.04 hours, respectively. The optimised solid lipid nanoparticle formulation's AUC 0-inf was 9805±0.45µg h/ml, which was much greater than the marketed drug dispersion's AUC0-inf of 4085±0.24µg h/ml. In comparison to marketed drug dispersion, the optimised solid lipid nanoparticle preparation's AUC0-t was statistically considerably greater ($p<0.05$). HPLC analysis of Nevirapine revealed that the retention periods for both Nevirapine and SE6 were 6.31 and 6.43 minutes, respectively. The standard calibration curve showed linearity with $R^2 = 0.999$ when plotted with concentrations ranging from 1 to 10 µg/ml.

CONCLUSION

In the current work, successfully formulated the solid lipid nanoparticles of poorly soluble drug Nevirapine which was optimized using design of experiment software by the process of high pressure homogenization technique. Formulation of in-situ gel was done after successful characterization and optimization of solid lipid nanoparticle dispersion.

The developed in-situ gel loaded with solid lipid nanoparticle of Nevirapine showed better absorption potential up to 14 times more effective with intranasal administration and 3.01 times more absorption upon oral administration of drug as compared to marketed oral dosage form availability.

Pharmacokinetic studies were carried out in adult male Wistar albino rats. Nevirapine Cmax of Nevirapine SLN 634±0.56µg/ml was more than that of the marketed drug (417±1.32µg/ml). Tmax of nevirapine SLN found to be 1.002±0.04 h while Tmax of marketed drug was in 2.10±0.05 h.

This proves that the optimized formulation of Nevirapine loaded in-situ gel provides better brain targeting that reduces the frequency of dose administration as well as the quantity of drug required to produce the desired actions. So the developed formulation upon necessary clinical trial investigations believes a great potential for the management of HIVs.

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