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# Fabrication and Evaluation of Amitriptyline HCl Loaded Nanostructured Lipid Carriers and In-Vitro Characterization

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**Abstract**

Both liquid and solid lipids make up the 2nd generation lipid nanoparticles, also known as nanostructured lipid carriers (NLCs), which are designed to increase medication encapsulation and storage stability. They enhance solubility, stability, and bioavailability. This study aims to develop an aqueous NLC dispersion for Amitriptyline HCl (AMT-HCl) to boost bioavailability while reducing dose frequency, amount, side effects, and toxicity.AMT-NLCs were prepared using the hot-melt high pressure homogenization (HPH) method, which contains Oleic acid as liquid lipid and stearic acid as solid lipids with a combination of Poloxamer 188 and Tween 80 as surfactants.The optimization involved adjusting the solid-to-liquid lipid ratio and surfactant concentrations. NLCs, which are advanced second-generation lipid nanoparticles, consist of both solid and liquid lipids, enabling improved drug encapsulation and reduced leakage during storage.AMT-NLCs evaluated for FTIR (no interaction), entrapment efficiency (77.3% to 84.4%), drug loading (9.9%) zeta potential (-33.7mv), particle size (93.33 nm), PDI (0.196), drug release (91.2%). TEM images showed AMT-HCl loaded NLC are in nano size with spherical in shape. Nanostructured lipid carriers (NLCs) prepared via the HPH method exhibited good entrapment efficiency, and particle size below 100 nm indicates better absorption through intranasal route and delivery of drug to the brain. The formulated batches demonstrated good drug loading and drug release pattern. Zeta potential indicates stable aqueous dispersion. The NLC (6) batch exhibited the most potential of all the produced batches, indicating that NLC may be the most effective approach for transporting medications to the brain through the intranasal route.NLC 6 holds promise for managing oxidative stress-related neurodegenerative diseases, including depression.

**Keywords: -** Amitriptyline HCl; depression; nose-to-brain; NLC; poloxamer 188; stearic acid.

## INTRODUCTION

Approximately 120 million people globally suffer from depression, a widespread mental illness characterized by low mood, loss of interest, reduced energy, disrupted sleep and appetite, and poor concentration. Depression is a significant public health concern especiallyyoung individuals are particularly affected, with an increase of up to 8% in depression rates. Suicide is the worst outcome of untreated major depressive disorder [1]. Alarmingly, over 50% of depressed patients don't respond to initial medication, and 30% remain symptomatic despite multiple treatments [2].

The blood-brain barrier presents a significant challenge in creating effective drugs for the brain, frequently resulting in unsuccessful treatments. [3,4,5,6,7].

Many patients with depression face difficulty swallowing conventional oral dosage forms due to dysphagia, especially children and the elderly. This poses challenges for treating mental disorders effectively [3]. Since the blood-brain barrier (BBB) acts as a biological filter, it frequently obstructs the passage of various curative agents, limiting the effectiveness of traditional drug delivery approaches[8].The blood-brain barrier (BBB) is lined with endothelial cells that contain ATP-binding cassette transporters, including P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). By acting as a protective shield, these proteins prevent pharmaceutical compounds from penetrating the central nervous system (CNS) via the bloodstream.Successful antidepressant therapy hinges on attaining adequate drug concentrations in the brain [9].

Delivering medications via the intranasal (IN) route provides a non-invasive and practical approach for targeting drugs directly to the brain.This method leverages the nasal cavity's rich blood supply, facilitating quick and effective absorption of the drug into the bloodstream and then into the central nervous system (CNS). The direct nasal route to the CNS ensures faster and more potent drug delivery than other methods. [10,11,12]. IN delivery enables drugs to directly access the brain through the olfactory and trigeminal nerve routes, circumventing the blood-brain barrier (BBB) and preventing complications such as gastrointestinal degradation and first-pass metabolism.This approach allows for faster drug absorption and a quicker onset of effects, resulting in higher drug levels in the brain while minimizing systemic side effects. Effective treatment of mental disorders relies on drugs crossing the BBB efficiently [13,14,15,16].

Over the last five years, several new studies have investigated the application of nanoparticles for delivering drugs from the nose to the brain, aiming to overcome issues with intranasal delivery. Methods like nanoemulsion [17], polymeric nanoparticles [18], liposomes [19], solid lipid nanoparticles (SLNs) [20] have emerged as effective systems for enhancing drug solubility and permeation, thereby improving nasal permeability and bioavailability for targeted brain delivery. These

approaches also help reduce mucociliary clearance and enzymatic degradation, enhancing drug efficacy [21,22].

The lipid-based particles known as nanostructured lipid carriers, or NLCs, have sizes ranging from 10 to 1000 nm. They were created in order to address the shortcomings of solid lipid nanoparticles (SLNs), particularly their low drug loading capacity and the possibility of drug leakage brought on by their crystalline nature.NLCs provide enhanced stability and longer shelf-life compared to SLNs [23]. NLCs provide extended drug release and enhanced stability, along with scalability using high-pressure homogenization, which is simpler compared to liposomes and nanoemulsion. These nanoparticles are created by blending long-chain solid fats with short-chain liquid fats, with typical mixing ratios falling between 70:30 and 99.9:0.1.This composition leads to a partially crystallized lipid structure that enhances drug entrapment [24]. Since nanostructured lipid carriers(NLCs) are flexible and nanosized, they can increase a drug's permeability through the nasal mucosa. This is because the lipid matrix protects the drug from enzymatic and chemical degradation, delays mucociliary clearance of the drug, and lengthens the nasal retention period. NLC-based formulations present significant benefits for transporting drugs from the nose to the brain without modifying the drug itself [25,26]. Research indicates that nanoparticles with a diameter smaller than 200 nm can effectively pass through the nasal passage and reach the brain. [27].

Amitriptyline hydrochloride (AMT-HCl), a commonly prescribed tricyclic antidepressant, is widely used for severe depression and anxiety. It works by inhibiting the reuptake of serotonin and norepinephrine at nerve endings. The typical daily adult dosage ranges from 75 to 150 mg. When taken orally, AMT-HCl is absorbed gradually and fully in the digestive system, reaching highest blood levels within 4-8 hours. When ingested orally, the drug is metabolized by the liver before entering the bloodstream, which leads to a systemic bioavailability of 33-62%.However, this drug may lead to unwanted effects like nausea, dry mouth, bad arrhythmias, and constipation due to its anticholinergic properties. Higher doses can also cause cardiac issues such as dysrhythmias and sinus tachycardia. Research indicates that a single dose of amitriptyline (75–100 mg) in healthy volunteers was poorly tolerated, suggesting that a gradual increase in plasma concentration is preferable. Clinical studies have shown that sustained-release formulations of AMT-HCl are more effective for both initiating and maintaining anticholinergic effects compared to immediate-release or standard dosage forms [28,29,30].

The research aimed to create and assess NLCs incorporating AMT-HCl using the Hot-Melt High-Pressure Homogenization technique. The objective was to address challenges associated with the drug by enhancing its bioavailability, minimizing dosing frequency and side effects, facilitating targeted delivery with prolonged drug release, and improving nose-to-brain delivery across the blood-brain barrier for improved therapeutic effects.

## MATERIAL AND METHODS

### Materials:

Amitriptyline HCl was purchased from Yarrow Chem Products (Mumbai, India); Stearic acid, glyceryl monostearate (GMS),Bees Wax, Oleic acid, Olive oil, Coconut oil PEG 400, Tween 80, sodium lauryl sulfate (SLS) and Poloxamer 188, as well as sodium chloride (NaCl), calcium chloride ($CaCl_2$), and potassium chloride (KCl), were obtained from Research-Lab Fine Chem Industries, Mumbai, India.

### Method:

#### Screening of Solid Lipids

In order to assess the excessive saturation solubility of AMT-HCl in solid lipids (stearic acid, GMS, and beeswax), 1 mg of the AMT-HCL was added to 1 g of melted solid lipid that had been heated in a water bath to 10°C above its melting point until drug saturation was achieved. After adding one ml of methanol, the lipid-drug combination was agitated for half an hour.Ultracentrifugation was performed for Ten minutes at 6000 rpm, and the aqueous phase was carefully collected. A one ml portion of the resulting transparent supernatant was mixed with Ten ml of methanol and examined for drug concentration by spectrophotometry at 242 nm[31].

#### Screening of Liquid lipid, Surfactants & Co-surfactants

To determine AMT-HCl's solubility in several liquid lipids (oleic acid, coconut oil, olive oil), surfactants, and co-surfactants (Tween 80, PEG 400, SLS, and Poloxamer 188) at excessive saturation, 1 mg of the AMT-HCl was added to 3 ml of each oil and surfactant in tiny glass vials. These vials were sealed, vortexed for 10 minutes, and left for 24 hours. After the specified duration, the samples were spun at 6000 rpm, and 1 ml of the clear supernatant was separated and mixed with 10 ml of methanol, then analyzed for drug concentration via spectrophotometry at 242 nm.[31].

#### Optimization of Ratios of Solid Lipid to Liquid Lipid:

To find the optimal balance between solid and liquid lipids for forming NLCs and to enhance drug loading, a solid-liquid lipid compatibility test was carried out. The test involved weighing the selected solid and liquid lipids in different proportions (1:1, 9:1, 8:2, 7:3, 6:4, and 5:5) for one hour, the physical mixture of SL and LL was heated to a temperature above 10°C, which is the melting point of SL. After pouring this substance onto the watch glass, it was chilled until it solidified completely. Next, a filter paper was applied on top of the solidified lipid melt, and the emergence of liquid lipid was evidenced by stains on the filter paper. This test identified the optimal ratio of solid lipid to liquid lipid based on their compatibility for NLC formulation. To achieve the highest drug loading, additional research was conducted by incorporating the drug in the ideal solid-to-liquid lipid ratio.The molten lipid blend in each vial was added with 5 mg of the medication in increments. The drug's complete dissolution, homogeneity, turbidity, and phase separation were then visually assessed. The mixture opted for the lipid phase in

the NLC design exhibited good miscibility and showed no signs of turbidity or phase separation [32,33].

**Preparation of AMT-HCl Loaded NLCs:**

Various AMT-HCl NLC formulations were made via the Hot Melt High-Pressure Homogenization (HPH) method shown in (fig 1). The solid lipid (stearic acid) was first heated to a temperature 10°C higher than its melting point using a magnetic stirrer. After heating, the liquid lipid (oleic acid) was added, and then AMT-HCl was carefully added and fully dissolved in the lipid mixture. The aqueous phase was prepared by dissolving a surfactant blend of Tween 80 and Poloxamer 188 (% w/w) in 200 ml of distilled water. The solution was then heated to match the temperature of the melted lipid phase.The lipid phase was then mixed into the heated aqueous surfactant solution while being stirred steadily at 1000 rpm for ten minutes to form a unrefined pre-emulsion (Remi 1 MLH Q-19, Mumbai, India).A high-speed homogenizer (IKA T25 digital ultra-TURRAX) was then used for mixing the pre-emulsion for Twenty minutes at 6000 rpm. After that, the resultant nanoemulsion was run through six homogenization cycles at 600 bars in a High-Pressure Homogenizer (Panda Plus 2000, GEA, Niro Soavi, Italy). After cooling the dispersion to ambient room temperature, it was maintained at 4–8°C overnight to assist in the recrystallization of the lipid and the formation of NLCs[27,31].

**Characterization of AMT-HCl Loaded NLCs Dispersion:**

**1. Drug, drug-excipient compatibility, and NLCs analysis by FTIR Spectroscopy**

The Fourier Transform Infrared (FTIR) spectrophotometer was used to assess the acceptability of AMT-HCl with the lipids included in NLCs. FTIR spectra were recorded for the pure drug, lipids, and AMT-HCl NLCs, with the scanning range spanning from 4000 to 400 $cm^{-1}$[34,35].

**2. Solubility Studies**

AMT-HCl's dissolution in various lipids was assessed by mixing the AMT-HCl and lipid in a vial, whirling the mixture until it was saturated, and then centrifuging the mixture at 6000 rpm to extract 1 ml of the clear supernatant solution Using spectrophotometric measurement at 242 nm, the drug concentration was then ascertained after this was diluted with 10 ml of methanol [32].

**3. Optical Microscopy**

A cover slip was placed over a sample of NLC dispersion that had been put on a glass slide. Next, using a pre-calibrated ocular micrometer, the sample was seen under a lab optical microscope to determine its average size and shape[36].

**4. Entrapment Efficiency (%EE) and Drug Loading (%DL)**

The encapsulation efficiency (EE) of AMT-HCl NLCs was assessed indirectly by centrifuging the sample, which allowed for the separation of drug-loaded nanoparticles from the aqueous phase that contained the unencapsulated drug.A cooling ultracentrifuge (Remi c-24) was used to centrifuge 10 mL of the dispersion at 10,000 rpmfor 30 minutes at 4°C. An UV spectrophotometer (Shimadzu – 1800, Shimadzu, kyoto, Japan) set at 242 nm was used to analyze the supernatant containing free AMT after it had been pipetted out and diluted with methanol in a ratio of 1:10% v/v. By utilizing the calibration curve of absorbance versus concentration of AMT-HCl, or the equation (y=mx+c), the amount of free drug contained in the transparent supernatant was further assessed. The resultant value was then entered into the calculation below [37].

$$EE\ (\%)=\frac{\text{Total amt of drug (mg)} - \text{Amtof free drug in supernatant (mg)}}{\text{Total amt of drug (mg)}} \times 100$$

$$DL(\%)=\frac{\text{Total amt of drug taken (mg)} - \text{Amt of free drug in supernatant (mg)}}{\text{Total amt of lipid (mg)}} \times 100$$

**5. Particle size (PS), Polydispersity Index (PDI), Zeta Potential (ZP)**

A Malvern Zetasizer (Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK) was employed to determine the particle size (PS) and polydispersity index (PDI) of the AMT–NLCs using dynamic light scattering (DLS).The analysis was conducted by diluting the NLC samples in a 1:10 (v/v) ratio with deionized water, and measurements were performed in disposable polystyrene cuvettes at 25 ± 2°C with a 90° scattering angle.The measurement lasted for more than 50 seconds while keeping the count rate close to 200 kcps[31,38]. The dispersant used was double-distilled water, which kept its refractive index (RI) at 1.33, viscosity at 0.8872 cP, and dielectric constant at 78.5 across every measurement. Zeta potential (ZP) was measured in folded capillary cells, with each sample also diluted with double-distilled water at a 1:10 (v/v) ratio[31].The samples underwent ultrasonication for 5 minutes prior to determining the particle size. Following this, the sample was placed in a single-use cuvette for measurement of particle size and zeta potential in the device[31].

**6. Transmission Electron Microscope (TEM)**

The structure of the refined AMT-HCl NLC formulation was analyzed using a Tecnai G2 Spirit BioTWIN transmission electron microscope (TEM) from FEI Company, located in Eindhoven, The Netherlands.To prepare sample for testing 0.1ml sample was diluted with 4 ml Distilled water in a glass vial. 0.5 ul of diluted sample is placed on 3 mm 200 mesh copper carbon grid then let it dry for ten min under Infrared lamp. After that stained with 10 µl of Phosphotungstic acid (PTA) is casted on the grid for 20 sec and remaining is wiped off using filter paper and the sample is analyzed under electron microscope. The grid is used for sample analysis at 120 kv.The images were captured at different magnification levels using the Soft Imaging Viewer software (Gatan Microscopy Suite-GMS3)[37,39,40].

**7. In vitro Drug Release Study**

The release of AMT-HCl in vitro was evaluated using the dialysis bag diffusion approach, by comparing the drug solution, a branded formulation, and the optimized AMT-HCl NLC formulation from three different suppliers.Each sample, comprising 5 mL containing 5 mg of the drug dissolved in simulated nasal fluid (SNF) with a pH of 5.95, was transferred into individual dialysis bags (HIMEDIA, molecular weight cutoff 12–14 KDa,

Maharashtra, India). Before being used, the dialysis bags were immersed in the SNF release medium for an entire night. To make the SNF, 8.192 mg/mL of NaCl, 0.4 mg/mL of $CaCl_2$, and 1.548 mg/mL of KCl were dissolved in water. The sealed dialysis bags containing the medication were suspended in 100 milliliters of 5.95 pH SNF, maintained at 37 ± 0.5°C, and agitated at 100 revolutions per minute. Sink conditions were maintained by taking 1 mL samples at certain intervals and replacing them with an equivalent volume of fresh medium. The concentration of AMT-HCl that permeated was measured using a UV-visible spectrophotometer, set at 240 nm, following filtration of the samples[27,39].

**8. Cell viability study**

The survival and proliferation of cells in the SH-SY5Y human neuroblastoma line were analyzed using the MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.) test.The cell suspension was brought to a concentration of $1.0 \times 10^5$ cells/mL using DMEM media with 10% FBS.In a 96-well flat-bottom microplate, 100 µL of this diluted cell suspension (at 70–80% confluence) was applied to each well. The cells were centrifuged after 24 hours, and the pellets were resuspended at four different concentrations (25, 50, and 100 µL) after they had attained a sufficient population density. Cells were checked every day during the 24-hour incubation at 37°C in an environment with 5% CO2.Following 24-hour period, the cells were exposed to 100 µM $H_2O_2$ for 90 minutes to provoke oxidative stress. MEM-PR (a version of MEM lacking phenol red) was mixed with 20 µL of MTT (2 mg/mL), and the plates were gently agitated.After incubation for two more hours at 37°C with 5% CO2, 100 µL of DMSO was gradually introduced to dissolve the formazan crystals, and the plates were lightly shaken. A microplate reader was then used to measure the absorbance at 540 nm.The dose-response curve helped in calculating the required concentration level and cell viability percentage to achieve 50% inhibition of cell growth. An Elisa microplate reader (Benesphera E21) was used to detect absorbance at 570 nm in triplicate samples. [41,42].

## RESULT AND DISCUSSION: -

**1. Screening of Solid lipids, liquid lipids and surfactants:**

The supersaturation method was employed to identify lipids and surfactants with the highest solubility for creating AMT-HCl NLCs. Among solid lipids, stearic acid showed the greatest solubility (77 mg/g) in (fig 2). In liquid lipids, oleic acid exhibited the highest solubility (89 mg/ml) in (fig 3), while Tween 80 was the most soluble surfactant for AMT-HCl (133 mg/ml) in (fig 4). Therefore, stearic acid, oleic acid, and Tween 80 were chosen for NLC preparation.

**2. Miscibility study:**

The study evaluated the potential for lipid separation or crystallization when combining solid and liquid lipids in varying proportions (1:1, 9:1, 8:2, 7:3, 6:4, and 5:5). Among these, the 7:3 ratio exhibited no evidence of separation or oil droplet formation and produced a smear on filter paper, signifying excellent miscibility. As a result, this ratio was chosen for the preparation of NLCs.

**Preparation and Optimization of AMT-NLCs:**

Several AMT-NLC formulations were created utilizing the hot melt high-pressure homogenization (HPH) technique, a productive, solvent-free procedure appropriate for manufacture on a laboratory scale and Industry (see Table 1). To optimize the physicochemical properties of the formulations, such as particle size (PS), polydispersity index (PDI), zeta potential (ZP), and entrapment efficiency (%EE), two key factorsthe surfactant blend concentration and the solid lipid-to-liquid lipid (SL: LL) ratiowere varied.A surfactant mix comprising Tween 80 and Poloxamer 188 was used to prepare the AMT-HCl NLC, with stearic acid and oleic acid acting as the solid and liquid lipids in a 7:3 proportion.

**Table no. 1 Formulation table for AMT-HCl loaded NLCs**

| Batch no. | Drug (mg) | Solid Lipid | Liquid Lipid | Tween 80 (ml) | PEG 400 (ml) | SLS (ml) | Poloxamer 188 (ml) | After 24 hrs |
|---|---|---|---|---|---|---|---|---|
| NLC-1 | 200 mg | 7 | 3 | 1.5 | 1.5 | - | - | Unstable |
| NLC-2 | 200 mg | 7 | 3 | 2 | 1.5 | - | - | Stable |
| NLC-3 | 200 mg | 8 | 2 | 1.5 | 1.5 | - | - | Unstable |
| NLC-4 | 200 mg | 8 | 2 | 2 | 1.5 | - | - | Stable |
| NLC-5 | 200 mg | 8 | 2 | - | - | - | 1.5 | Phase separation |
| NLC-6 | 200 mg | 7 | 3 | 2 | - | - | 1.5 | Stable |
| NLC-7 | 200 mg | 8 | 2 | - | - | - | 2 | Phase separation |
| NLC-8 | 200 mg | 8 | 2 | 1.5 | - | 1.5 | - | Phase separation |

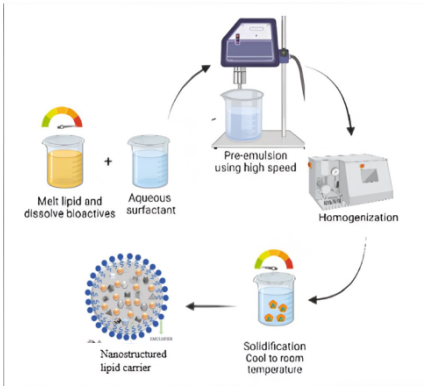


**Fig 1. Preparation of NLCs using Hot-melt High Pressure Homogenization**

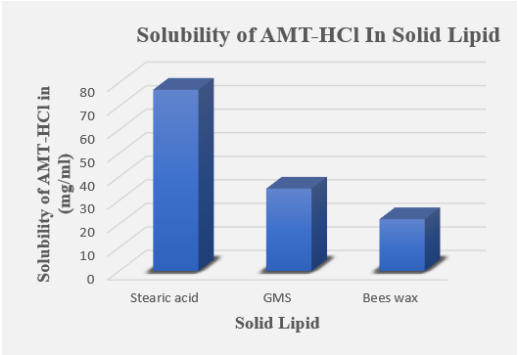


**Fig 2: Solubility of AMT-HCl in different (A) Solid lipids**

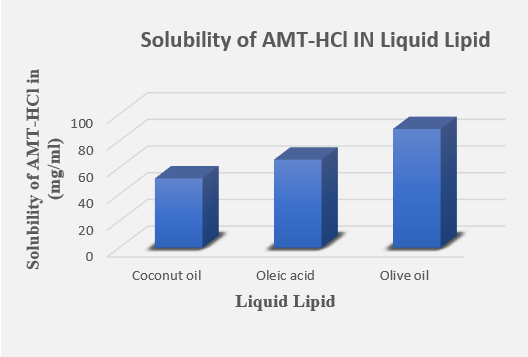


**Fig 3: Solubility of AMT-HCl in different (B) Liquid Lipids**

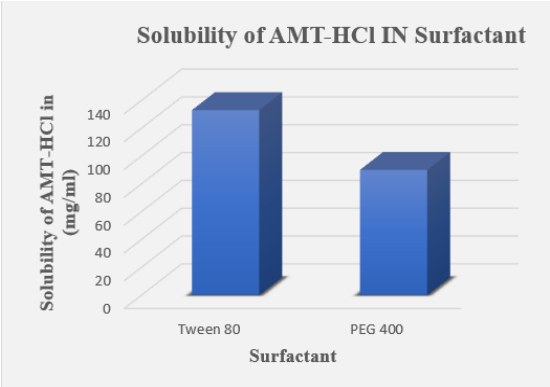


**Fig 4: Solubility of AMT-HCl in (C) Surfactants**

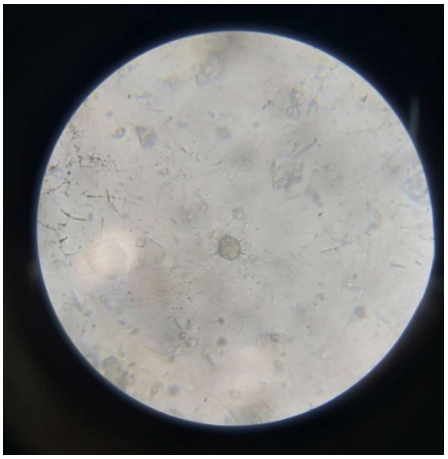

**Fig 5. The optical microscopic images of NLC 6 (A) Before HPH**

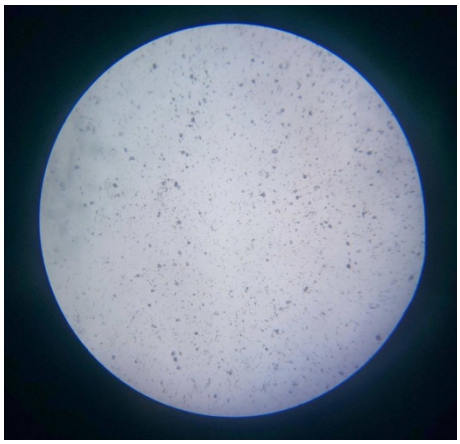

**Fig 6. The optical microscopic images of NLC 6 (B) After HPH**

## 3. Optical microscopy:

In this microscopy we aim to elucidate nanostructured lipid carriers (NLCs) structural characteristics and morphology at the nanoscale level, potentially revealing insights into their stability and drug delivery properties with the difference between particle size analysis before and after the use of high-pressure homogenization (HPH). Upon observing the microscopic images, we anticipate identifying distinct lipid bilayer formations encapsulating drug payloads, assessing particle size distribution, possibly uncovering any aggregation tendencies and the difference between particle size in image A in (fig 5) and B in (fig 6).

## 4. Thermodynamic stability of NLCs:

The formulations, as presented in Table 2, were exposed to a freeze-thaw cycling test (spanning from -4°C to 40°C for 24 hours over 7 days), and their stability was maintained under these conditions.

**Table no. 2 Results of thermodynamic stability of NLCs**

| Batch no. | Results |
|---|---|
| NLC 2 | Phase Separation |
| NLC 4 | Phase Separation |
| NLC 6 | Stable |

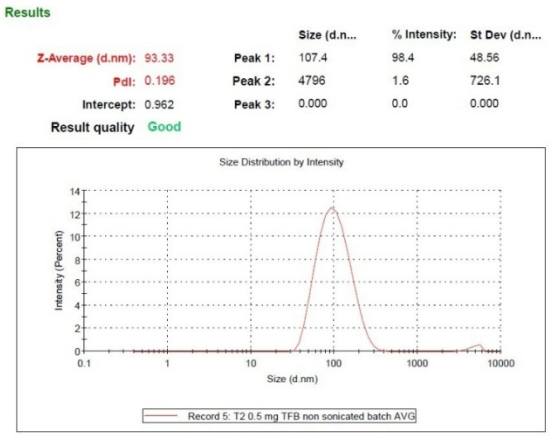


**Fig 7. Images of NLC 6 (A) Particle size distribution**

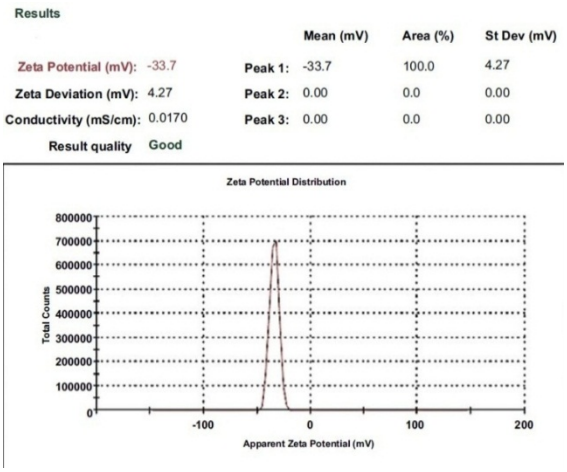


**Fig 8. Images of NLC 6 (B) Zeta potential**

## 5. Entrapment Efficiency (%EE) and Drug Loading (%DL):

The other three stable formulations in Table 3 exhibited the highest drug loading (9.9%) and encapsulation efficiency (84.4%) when compared to the NLC (6) formulation. The presence of both liquid and solid lipids in the NLC structure disrupts its crystal lattice, allowing more room for the inclusion of extra drug molecules (AMT-HCl), thereby increasing the EE[27].

**Table no. 3 Entrapment efficiency and drug loading of NLCs**

| Batch no. | Entrapment Efficiency | Drug Loading |
|---|---|---|
| NLC 2 | 81.87% | 10.22 |
| NLC 4 | 77.3% | 8.25% |
| NLC 6 | 84.4% | 9.9% |

## 6. Particle size (PS), Polydispersity Index (PDI), Zeta Potential (ZP):

Nanocarriers' biopharmaceutical characteristics, such as the speed of drug release, absorption, and how the drug is distributed in the body, are heavily influenced by the particle size.The drug may penetrate the nasal mucosa more effectively and reach the brain more quickly due to its reduced size. This could also enhance the drug's delivery and absorption into the brain[27].The optimized batch of AMT-HCl NLC (6) exhibited a mean particle size of 93.33 nm, which is below 200 nm, making it suitable for brain targeting via the intranasal route.

The PDI, or Polydispersity Index, plays a key role in evaluating the eveness of a dispersion, showing whether it is monodisperse or polydisperse and pointing to the likelihood of aggregation of particles for preventing clumping and ensuring uniform nanoparticle distribution, a lower PDI value is preferred [27]. Higher PDI values suggest a broader size distribution, while lower values indicate a more uniform particle population. The optimized NLC 6 batch demonstrated a PDI of 0.196, signifying a narrow particle size distribution, which is below the ideal threshold of 0.3. The Zeta potential (ZP) measures the electrical charge on the surface of NLC and provides insight into the formulation's stability by indicating the likelihood of particle aggregation. A ZP value greater than +30 mV or less than -30 mV typically signifies good stability, as it prevents particle clustering [27]. The optimized NLC 6 formulation exhibited a ZP of -33.7 mV, indicating outstanding stability.These results are shown in (fig7 & 8).

## 7. Transmission Electron Microscope (TEM):

TEM analysis of the optimized AMT-HCl NLC (6) (fig 9) formulation showed spherical nanoparticles with smooth surfaces, ranging in size from 10 to 100 nm, and no signs of aggregation.

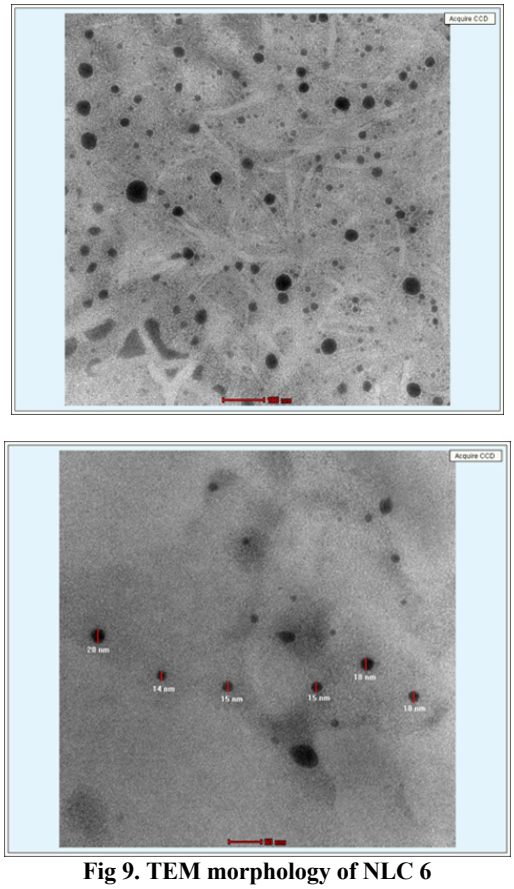

**Fig 9. TEM morphology of NLC 6**

**Table no. 4 Effects of NLC-6 against SH-SY5Y Neuroblastoma Cell Line by MTT assay**

| Concentration (µL) | Absorbance (O D) | | | | Cell viability (%) | Cell inhibition (%) |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | Average | | |
| **Control** | 1.207 | 1.191 | 1.2 | 1.199333 | | |
| **H2O2** | 0.175 | 0.181 | 0.167 | 0.174333 | 14.5358533 | 85.4641467 |
| **NLC (6) – 25** | 0.511 | 0.531 | 0.527 | 0.523 | 43.6075598 | 56.3924402 |
| **50** | 0.487 | 0.477 | 0.48 | 0.481333 | 40.1334074 | 59.8665926 |
| **100** | 0.451 | 0.462 | 0.458 | 0.457 | 38.1045025 | 61.8954975 |

## 8. *In-Vitro* Drug Release Study:

A biphasic release pattern is revealed by the release profile of AMT-HCL from the drug solution, the commercial formulation, and the improved AMT-HCl NLC (6) formulation, as shown in
(fig 10). The improved formulation showed a slow and consistent release of 91.2% over the course of 8 hours, after an initial burst release of 18.7% during the first hour.This biphasic release pattern observed in Nanostructured Lipid Carriers (NLCs) is likely caused by the heterogeneous composition of solid and liquid lipids within the NLC matrix. The prolonged release is attributed to drug embedded within solid lipid crystals, while the initial rapid release is likely due to drug in the liquid lipid phase, where diffusion occurs more quickly than in the solid lipid phase [27]. whereas, drug release by pure drug solution and marketed formulation showed initial release of 15.1% and 8.2% in first hour and 85.5% and 79.1% up to 8 hrs respectively, both drug solution and marketed formulation shows fluctuation in drug release when compared to optimized formulation this leads to poor therapeutic outcome hence, NLC (6) formulation shows stable drug release pattern which provides better therapeutic outcome.

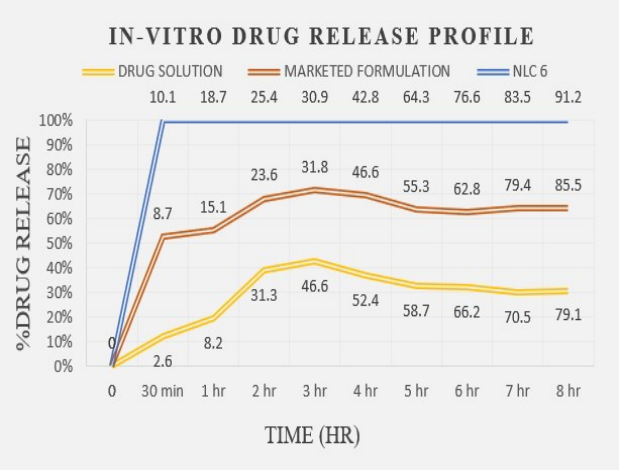


**Fig 10. Graphical representation of In-vitro drug release of NLC 6**

## 10. Cell viability study:

The data reveal a significant decrease in SH-SY5Y cell growth that is directly related to higher concentrations of reactive oxygen species (ROS), triggered by oxidative stress. The compound NLC 6 contains antioxidant components and exhibits neuroprotective effects, effectively reducing ROS levels and inhibiting cell proliferation in SH-SY5Y cell lines shown in (Table no. 4). Therefore, NLC 6 holds promise for managing oxidative stress-related neurodegenerative diseases, including depression.

## CONCLUSION

The nanoparticle drug delivery system offers an effective strategy for targeting brain-related conditions. Among various techniques, hot melt high-pressure homogenization (HPH) is considered the most efficient, solvent-free, and scalable method for preparing nanostructured lipid carrier (NLC) dispersions. In this research, an NLC dispersion containing Amitriptyline HCl (AMT-HCl) was successfully developed using stearic acid and oleic acid in a 7:3 ratio, combined with a surfactant mixture of tween 80 and poloxamer 188. During freeze-thaw cycles, the improved NLC formulation (batch 6) demonstrated stability with an entrapment effectiveness of 84.4% and a drug loading of 9.9%. With a polydispersity index (PDI) of 0.196 and an average size of 93.33 nm, the NLC particles were suitable for intranasal drug administration. The stability of the NLC dispersion was confirmed by its Zeta potential of -33.7 mV, and transmission electron microscopy (TEM) pictures revealed that the AMT-HCl-loaded NLC particles were nano-sized and spherical. Furthermore, when compared to a commercial formulation and a pure drug solution, the NLC dispersion showed a sustained drug release profile, with 91.2% release over 9 hours.In in-vitro cell line studies, NLC batch 6 showed potential for managing oxidative stress-related neurodegenerative diseases, such as depression. These findings imply that AMT-HCl-loaded NLC dispersion may be a useful nasal medication delivery method for the brain. Through intranasal administration, this work may help researchers create in-situ gelling devices for brain medication delivery, which would eventually improve treatment results and drug bioavailability.

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