BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, September 2024. Vol. **21**(3), p. 1145-1156

Development of Nanostructured Lipid Carriers for Donepezil Hydrochloride Effective Nose to Brain Delivery

Rohit Thete, Ganesh Shevalkar and Laxmikant Borse

Department of pharmaceutics, Sandip Institute of Pharmaceutical Sciences, Nashik, Maharashtra, India.

http://dx.doi.org/10.13005/bbra/3293

(Received: 29 July 2024; accepted: 24 September 2024)

This study focuses on the development and evaluation of nanostructured lipid carriers (NLCs) for the efficient intranasal delivery of donepezil hydrochloride. The NLCs were prepared using the microemulsion technique. Each excipient used in the formulation was thoroughly evaluated for stability, assessing factors such as color change, phase separation, precipitation, and texture. Characterization of the NLCs included the construction of a pure calibration curve, differential scanning calorimetry (DSC), infrared spectroscopy (IR), and the IR analysis of physical mixtures. The donepezil hydrochloride-loaded NLCs were then incorporated into a thermosensitive gel using Pluronic F127, which was also prepared using the microemulsion technique. The NLCs were further characterized by evaluating their zeta potential, entrapment efficiency, and particle size. The thermosensitive gel's properties were assessed by measuring the gelation temperature and viscosity. Drug release studies were conducted using a dialysis membrane to compare the release profiles of the pure drug, donepezil-HCl-loaded NLCs, and the NLC-loaded thermosensitive gel, focusing on their potential for controlled drug release.

Keywords: Donepezil Hydrochloride; Nano formulation; NLCs; Thermosensitive gel.

Nasal-to-brain drug delivery is an innovative and non-invasive approach for administering therapeutic agents directly to the brain via the nasal cavity. This method allows drugs to bypass the blood-brain barrier (BBB), a significant challenge in treating central nervous system (CNS) disorders. The BBB serves as a protective barrier that restricts most drugs from passing from the bloodstream into the brain, thereby limiting the attainment of effective therapeutic concentrations within the CNS.[1] The nasal route provides a direct path to the brain, which can potentially enhance the management of neurodegenerative diseases, brain tumors, and psychiatric disorders. The nasal cavity utilizes two main pathways for delivering drugs to the brain: the olfactory pathway and the trigeminal nerve pathway. The olfactory region, located at the top of the nasal cavity, contains sensory neurons that connect directly to the brain's olfactory bulb. In contrast, the trigeminal nerve pathway involves sensory neurons within the nasal mucosa that extend into the brainstem. Both pathways enable drugs to bypass the BBB, allowing for more effective delivery to the central nervous system (CNS).[2]

*Corresponding author E-mail: rohitthete93@gmail.com

Alzheimer's disease is a progressive neurodegenerative condition characterized by cognitive decline, memory impairment, and behavioral changes. It is the leading cause of dementia in the elderly population. The pathophysiology of Alzheimer's involves the accumulation of amyloid-beta plaques and tau tangles in the brain, leading to neuronal damage and brain shrinkage. The disease progresses through several stages, beginning with mild cognitive impairment and advancing to severe dementia, greatly affecting the patient's quality of life and independence.[3] The development of Alzheimer's disease is influenced by various factors, including genetic predisposition, environmental influences, and lifestyle choices. Key genetic risk factors include mutations in genes such as presenilin 1 (PSEN1), amyloid precursor protein (APP), and presenilin 2 (PSEN2), along with the presence of the apolipoprotein E (APOE) å4 allele. Environmental and lifestyle factors, such as cardiovascular health, diet, physical activity, and educational background, also play a significant role in the onset and progression of the disease.[4]

Nanostructured Lipid Carriers (NLCs) are a type of nanoparticle used in drug delivery applications. These carriers are composed of a combination of solid and liquid lipids, forming a unique nanostructure capable of encapsulating lipophilic, hydrophilic, and amphiphilic drugs. NLCs offer several advantages over traditional drug delivery systems, including improved drug stability, controlled release, and enhanced bioavailability. Their biocompatibility and ability to overcome physiological barriers make them particularly valuable for treating conditions such as cancer, infections, and neurodegenerative diseases.[5] The composition of NLCs, including lipid content, surfactants, and manufacturing methods, can be tailored to optimize drug delivery. This flexibility allows for the development of personalized medicine strategies, customizing the drug delivery system to meet the specific needs of individual patients. In the context of Alzheimer's disease, NLCs are being explored for their potential to deliver therapeutic compounds directly to the brain, overcoming the challenges posed by the blood-brain barrier (BBB).[6]

Thermosensitive gels are a specialized type of hydrogel that undergoes a reversible sol-gel phase transition in response to temperature changes. These hydrogels remain in a liquid state at lower temperatures but transition to a gel state at physiological temperature (around 37°C). This unique property makes thermosensitive hydrogels particularly attractive for various biomedical applications, including drug delivery, tissue engineering, and wound healing. In drug delivery, thermosensitive hydrogels offer the advantage of being injectable in their liquid form, allowing for easy administration. Once at the target site, they undergo gelation, enabling sustained and localized drug release. This targeted approach helps minimize systemic side effects and enhances the therapeutic effectiveness of the treatment. Commonly used polymers in the development of thermosensitive hydrogels include poly(N-isopropylacrylamide) (PNIPAAm), Pluronic F127, and chitosan-based polymers.[7,8].

MATERIAL AND METHOD

Materials

Donepezil hydrochloride sourced from Dr. Reddy's Laboratories Ltd. in Hyderabad, India. Gelucire 43/10[9] obtained from Gattefossé. Capmul MCM[10] and methanol was acquired from Abitec Corporation. Creomophore RH 40[11], polysorbate 80, and Pluronic F127[12] was purchased from BASF. PEG and Benzyl alcohol were obtained from Sigma Aldrich. Chitosan was sourced from Primex. All other solvents and ingredients utilized were of analytical grade.

Characterization of Donepezil HCL

Preparation of stock solution

To prepare a 1000 ppm solution, 10 mg of the active pharmaceutical ingredient (API) was added to 10 ml of solvent in a 10 ml volumetric flask. Subsequently, 1 ml of the 1000 ppm solution was pipetted into another 10 ml volumetric flask and diluted to the mark with solvent to obtain a 100-ppm solution. For the calibration curve, readings were taken at concentrations of 20, 40, 60, 80, and 100 ppm. The analysis was conducted using UV spectroscopy.[13]

Fourier transfer infrared Spectroscopy

ATR-IR spectroscopy provides valuable insights into the structure and functional groups present in chemical compounds. In this study, the interaction between the drug and excipients

was examined using ATR-IR spectroscopy. The IR spectra of the pure drug, physical mixture, and formulation were obtained using an ATR-IR instrument (Perkin Elmer), with 1–2 mg of the sample placed on the holder and scanned over a range of 550–4000 cm{ ¹. The resulting spectra were analyzed for any changes, with particular attention to the characteristic peaks corresponding to the functional groups in the sample compounds.[14]

DSC (Differential scanning calorimetry) Analysis

DSC (Differential scanning calorimetry) is a thermal analysis technique used to measure the heat input or output of a sample using a calorimeter. In the case of Donepezil hydrochloride, DSC thermograms were used to analyze the drug. The interaction of the drug is indicated by the appearance of new peaks, the absence of endothermic or exothermic peaks, deviations in the shape of standard peaks, or alterations in peak characteristics. The samples, each weighing 5 mg, were placed in standard aluminum pans and sealed. using a DSC 60 Plus, SHIMADZU The samples were then heated at a constant rate of 10 °C/min over a temperature range of 25°C to 300°C, and the resulting DSC spectra were recorded[15].

Preformulation study (For solubility For Formulating NLCs)

Solubility of Solid lipids

The determination of DNP HCL solubility in solid lipids involved the solubilization of DNP HCL in molten lipid Precirol ATO 5, Geleol, Gelucire 43/10. To begin, an accurate measurement of 1 g of solid lipid was then transferred to a test tube. The test tube was then heated on a water bath, maintaining a temperature at least 10 °C higher than the MP of the solid lipid. Incrementally, DNP HCL was added to the molten lipid in 2 mg portions and mixed vigorously using a cyclomixer (Remi, CM 101). The solubility of DNP HCL was evaluated by visually inspecting for the presence or absence of drug crystals. The point at which the drug no longer exhibited any solubilization was considered as the endpoint for the drug's solubility[16].

Solubility of liquid lipids

In short, an excessive amount of the drug was introduced into 1 gram of chosen liquid lipid like Capmul MCM, Oleic acid, Iso-propyl myristate in Eppendorf tubes with a capacity of 2 mL and agitated using a cyclomixer from Remi Instruments, India. Subsequently, these tubes were placed in a temperature-controlled water bath shaker from Remi Instruments, India, set at 25 ± 1 °C for a duration of 72 hours. Following this, the tubes were spun at 10000 rpm for 15 minutes using a Microcentrifuge from Remi[16], RM-12C. The resulting liquid above the sediment was then isolated, and samples were extracted and appropriately diluted with methanol. Take reading with the help of UV Spectroscopy

Formulation of NLCs and NLC loaded thermosensitive gel

Method of preparation of NLCs

Accurately weighing Solid lipid, liquid Lipid and Surfactant in Test tube. Heat The Test Tube with The Help of Induction Until It Get Melt Down. Add the API And Shacked with the help of vortex shaker. Add stabilizer into aqueous phase. Mix the both and shake with the help of vortex shaker. Shaked Continuously until proper mixing. Formed pre-emulsion was sonicated by probe-type sonicator (15 min, 40W, pulse: 6 s ON and 3 s OFF) for particle size reduction. Cool Down Solution and Do the Dispersion in Different media. Batches Showed in Table no.1[16].

Method of preparation of NLCs loaded TSG

Placed the distilled water in a refrigerator to cool it to approximately 4°C. Weigh 2 g of Pluronic F-127. Slowly add Pluronic F-127 powder and PEG to cold water while continuously stirring with a glass rod. Add slowly NLCs (2 ml) to the Pluronic F 127 solution while stirring. Ensure that it gets uniformly dispersed. Add chitosan and benzyl alcohol to the surface of the solution with consciously stirring. Store the thermosensitive gel at 4°C to maintain its liquid state until use. Batches Showed in Table no.2.2[17–20].

Characterization of developed NLCs and NLCs loaded thermosensitive gel

Entrapment Efficiency

Drug entrapment efficiency

The ultracentrifugation method was used for the determination of EE (entrapment efficiency). 2 mL of the DNP HCL loaded NLCs and NLCs loaded thermosensitive gel dispersion filled in centrifuge tubes and ultracentrifugation was carried out at 35,000 rpm for 2 h (Microcentrifuge, RM-12C). The cleared supernatant liquid contained un-entrapped drug was removed out and filtered through a 0.45 ìm syringe filter. It was then

diluted appropriately with methanol followed by UV analysis. The EE (entrapment efficiency) was determined used the followed equation[16].

% Entrapment efficiency = [Total amount of drug added - Amount of drug in supernatant / Total amount of drug added] × 100

Determination of particle size

The DNP HCL NLCs and Thermosensitive gel were analyzed for particle size, PDI, and zeta potentials using a particle size analyzer. The particle size was measured at a scattering angle of 90° and a temperature of 25°C. To conduct the measurement, 1 ml of NLCs and Thermosensitive gel solutions was diluted with 10 ml of distilled water while being stirred constantly by a magnetic stirrer for 1 minute. The resulting solution was then analyzed for particle size using dynamic light scattering technique with a Zetasizer (Nano ZS, Malvern Instruments, UK)[21–23].

Zeta Potential Determinations

The laser diffraction analysis technique was used to determine the zeta potential of the optimized preparation, employing a particle size analyzer (Malvern Zetasizer Nano Series ZS 90). The NLCs and Thermosensitive gel formulation, each weighing 1 mg, were diluted with 10 ml of water(distilled) and stirred for 1 minute using a magnetic stirrer at a temperature of 25.2^0C. All experiments were conducted in triplicate[10,24,25].

Gelation Temperature

In order to assess the gelation of NLCs-loaded thermosensitive gel, it was necessary to create samples of gelatin solution and distribute them evenly into small vials. These vials were then sealed and turned upside down to allow air bubbles to gather at the bottom. Subsequently, the vials were placed in an environment with controlled temperature, where the temperature was gradually raised until the gelation temperature was reached, indicating the transition of the substance from liquid to gel. To evaluate viscosity, the gel formulations were stored at 4°C and their viscosity was measured using a viscometer to ensure a liquid consistency suitable for easy administration. Following this, the formulations were heated to 37°C and viscosity was measured again to confirm the transition to a semi-solid state, which was ideal for nasal retention and sustained release purposes. Finally, it was important to meticulously document and analyze the obtained results[26–28].

Viscosity studies

Viscosity assessment of thermosensitive gels loaded with intranasal NLCs is essential. By conducting measurements at both 4°C and 37°C, the study guarantees convenient application and appropriate gel formation. The gel maintains a liquid state at 4°C, facilitating easy administration. Conversely, at 37°C, it transitions into a semi-solid

Table 1. Formulation batches NLCs

Sr. No.	Chemical name	F1	F2	F3	F4	F5	F6
1.	DNP HCL (mg)	10	10	10	10	10	10
2.	Gelucire 43/10 (mg)	200	100	200	200	100	200
3.	Capmul mcm (mg)	300	300	150	300	150	150
4.	Cremophor RH 40 (mg)	300	200	200	200	200	300
5.	Polysorbate 80 (mg)	0.2	0.1	0.3	0.2	0.4	0.3
6.	Water (ml)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table 2. Formulation Batch TSG

Chemicals	Percentage	Role of Ingredient
NLCs DNP	50 % v/v	vehicle
Pluronic F127	40 % w/v	Thermosensitive gelling agent
PEG	9 % w/v	Solubilizing agent
Chitosan	0.2 % w/v	Mucoadhesive agent
Benzyl Alcohol	0.8 % w/v	Preservative

state, promoting nasal retention and prolonged drug release. This validates the reliability of the formulation, with the assistance of a Brookfield viscometer for analysis[10,21,23].

In-vitro drug release profile

In order to initiate the dialysis membrane experiment, the membrane underwent a 12-hour immersion in distilled water followed by a rinse with PBS. Subsequently, NLC and NLC-loaded thermosensitive gel were each inserted into separate sealed dialysis bags. The release medium was created by filling two beakers with PBS at 37°C, utilizing a magnetic stirrer or incubator. The dialysis bags were then submerged into the PBS solution and agitated at 100 rpm. At specified time intervals (ranging from 2 to 24 hours), a fixed volume of the release medium was withdrawn and replaced with fresh PBS to uphold sink conditions. The concentration of drug was determined through UV-Vis spectrophotometry at 315 nm to assess the drug release profile from both NLCs and NLCs loaded thermosensitive gel[10,29,30].

Nasal permeation study

To prepare nasal tissue from a Deccani sheep, excise and fit the nasal mucosa into Franz diffusion cells, preserving the epithelial surface. Rinse with PBS to remove mucus. Position the tissue in the Franz cell, mucosal side towards the donor compartment, and seal to prevent leakage. Equilibrate at 37°C for 30 minutes. Introduce the NLCs-loaded thermosensitive gel in the donor compartment and fill the receptor compartment with PBS, maintaining 37°C and continuous stirring. Collect samples from the receptor at specified intervals (0, 2, 4, 6, 8, 12 hours), replenishing with fresh buffer. Analyze drug concentration via UV-Vis spectrophotometry to evaluate permeation[31].

RESULTS

IR Spectroscopy- DNP HCL

The DNP HCL pure drug displays functional peaks at 2961.67 cm-1, confirming

Table 3. Particle Size, PDI and ZP – Batch-F4

Title	Particle Size(nm)	Entrapment efficiency (%)	Zeta potentiometer	PDI
DNP-NLC	228.5	86.66	-31.42 mV	0.210
Thermosensitive gel	228.9	86.70	-30.32 mV	0.225

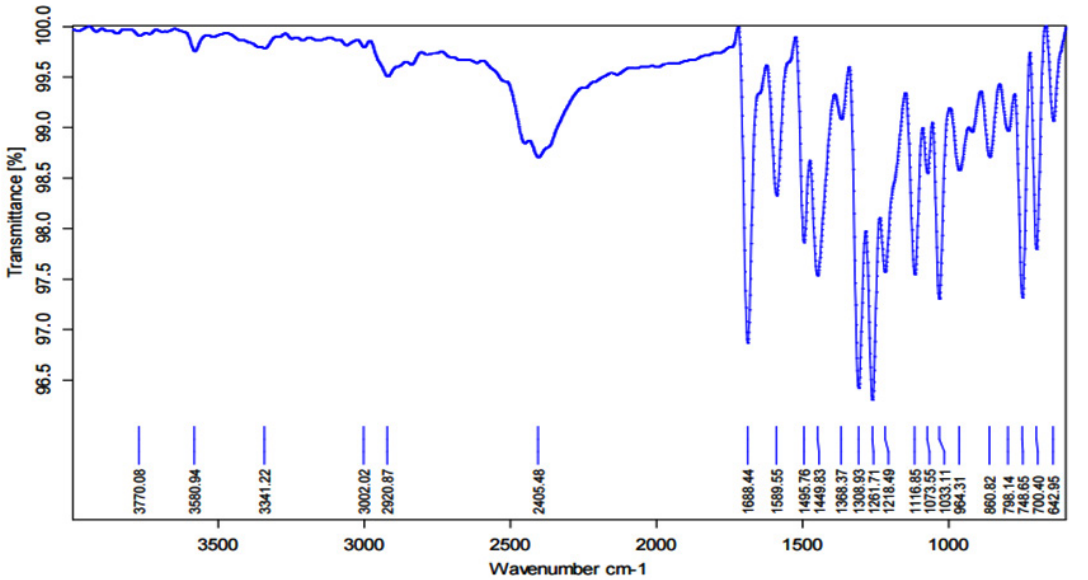

Fig. 1. IR Spectra of DNP HCL

the existence of Aromatic and Aliphatic C-H Stretching. Additionally, peaks at 3002.02, 2920, 1368, and 700 indicate the functional groups C=O, Ring C-N, C-S, C-O-C, and Para di substituted aromatic ring, respectively (Table). Based on the frequencies observed, it can be concluded that Donepezil HCl is a pure drug.

DSC Of Donepezil HCL

The thermal behavior of pure Donepezil hydrochloride was assessed by analyzing the DSC thermogram presented in the figure 3.5

The results of the DSC analysis of Donepezil hydrochloride suggest that the Donepezil hydrochloride used in the preparation of NLC loaded Thermosensitive gel is pure. The melting point of Donepezil hydrochloride was recorded as 228.94°C, with an onset at 227.89°C and an end set at 236.47°C. The presence of a distinct endothermic peak indicates that Donepezil hydrochloride has crystalline properties.

Solubility in Solid lipid ,liquid lipid and surfactant

Solid Lipids: Precirol ATO 5 (10 ± 2 mg/g), Geleol (8 ± 2 mg/g), Gelucire 43/10 (15 ± 2

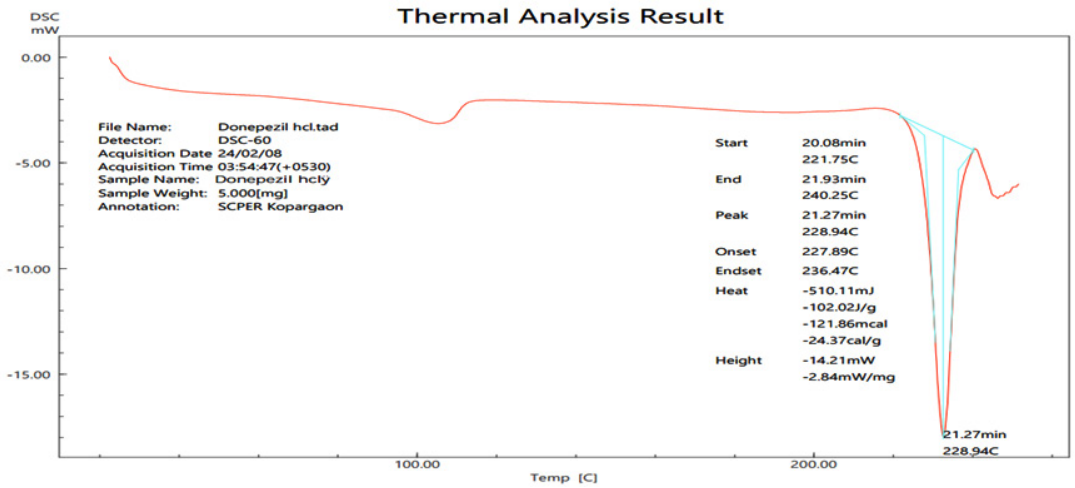

Fig. 2. DSC Thermogram Of Donepezil HCL

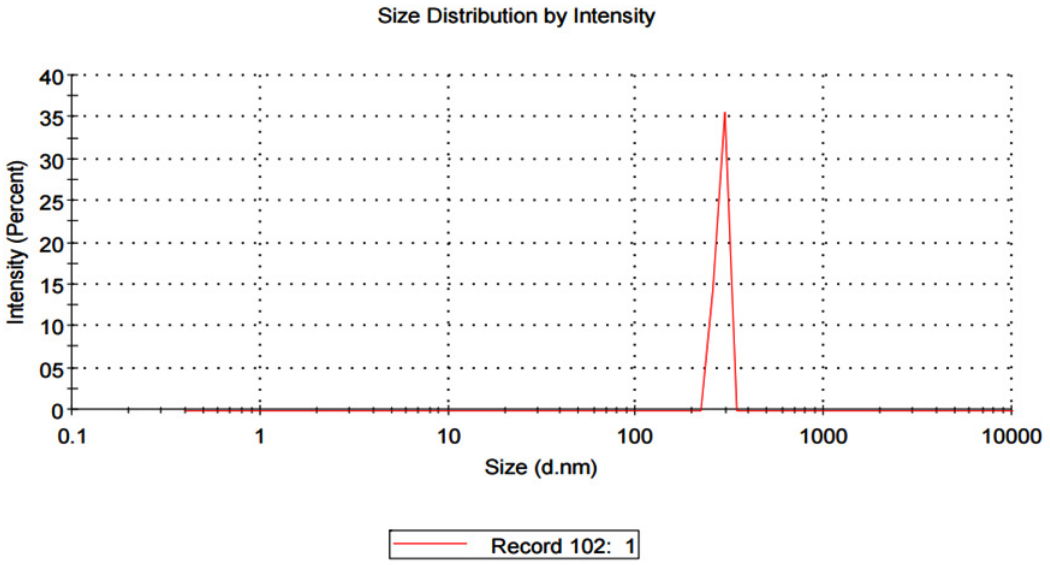

Fig. 3. Particle size of NLC (Batch-4)

mg/g). Liquid Lipids: Capmul MCM (45 ± 5 mg/g), Oleic Acid (20 ± 5 mg/g), Isopropyl Myristate (15 ± 5 mg/g). Surfactants: Kolliphore RH 40 (1 ± 1 mg/g), Tween 80 (2 ± 1 mg/g).

Particle Size, PDI and ZP – Batch-F4

Particle size of NLC and Thermosensitive gel

The particle size distributions of two samples were analyzed using a Malvern Panalytical Zetasizer on June 27, 2024. The results from FIG 8.8 (Record 102) indicated a primary peak at 283.7 d.nm with 100% intensity, a Z-average size of 228.9 d.nm, and a PdI of 0.210, suggesting a narrow size distribution. On the other hand, FIG 8.9 (Record 104) displayed a primary peak at 238.9 d.nm with 60.2% intensity, as well as a secondary peak at 70.2 d.nm with 9.8% intensity. The Z-average size was 228.5 d.nm, and the PdI was higher at 0.255, indicating a broader size distribution. It is worth noting that both measurements were conducted under consistent conditions, with 170 kcps and at a temperature of 25°C.

Entrapment efficiency (%)

Entrapment efficiency is a crucial parameter in drug delivery systems, as it indicates the proportion of the drug successfully encapsulated within the delivery vehicle. In the context of nanostructured lipid carriers (NLCs) and thermosensitive gels, high entrapment efficiency can be attributed to several factors.

Nanostructured Lipid Carriers (NLCs)

- Lipid Matrix[32]
- High Lipophilicity[33]
- Surface Properties[33]

Thermosensitive Gel

- Controlled Release[7]
- High Viscosity[34]
- Biocompatibility[35]

Table 4. Viscosity of NLC loaded thermosensitive gel

Batches	pH	Viscosity (cp) at 4°C	Viscosity (cp) at 37°C
F1	6.1	21	5204
F2	6.2	28	5365
F3	6.1	21	5269
F4	5.9	17	5026
F5	6.1	23	5199
F6	6.0	26	5120

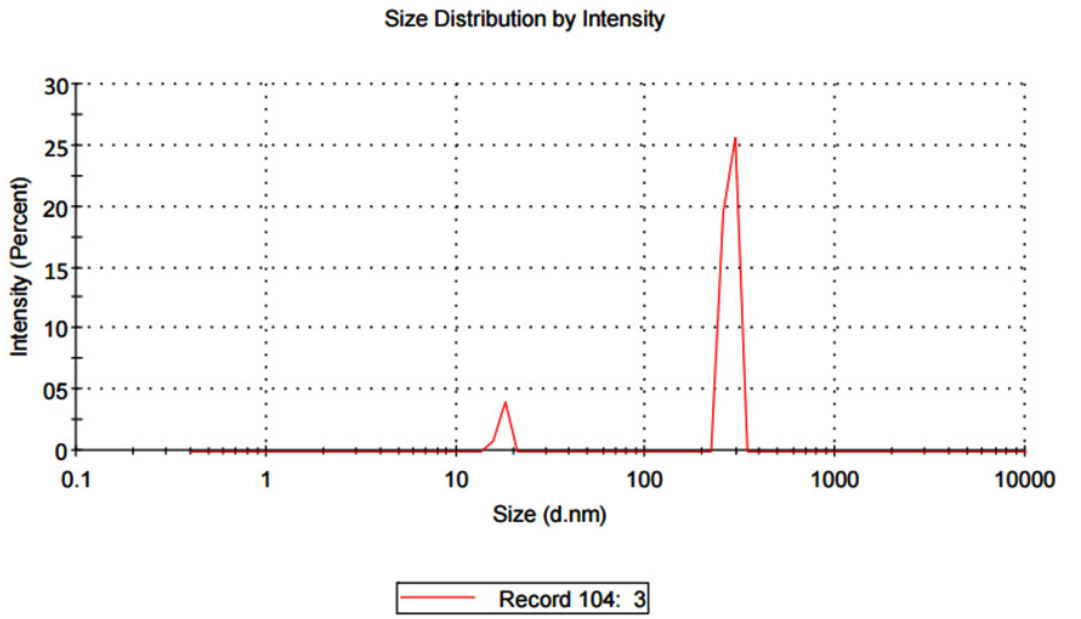

Fig. 4. Particle size of thermosensitive gel (Batch-4)

Formulated NLCs and Thermosensitive gel of Batch F4 have showed entrapment efficiency. NLCs Showed 86.66 and 86.70 percent.

Zeta potential Of NLCs and NLCs loaded Thermosensitive gel F4-Batch

Zeta potential measurements for a thermosensitive gel sample were conducted using a HORIBA SZ-100 on May 24, 2024. The sample at 25.2°C in a dispersion medium with 0.894 mPa·s viscosity and 0.218 mS/cm conductivity showed a zeta potential of -30.32 mV and electrophoretic mobility of -0.000245 cm²/Vs, indicating net negative surface charge and stability. Another measurement at 25.1°C with the same dispersion medium showed a zeta potential of -31.42 mV and electrophoretic mobility of -0.000285 cm²/Vs, also indicating net negative surface charge and stability in the dispersion medium.

Gelation temperature

Recorded the temperature at which gelation occurs for each sample tube. The result shows at 37.3 °C temperature. The NLC-loaded thermosensitive gel transitions from liquid to gel at 37.2°C, allowing easy application and prolonged

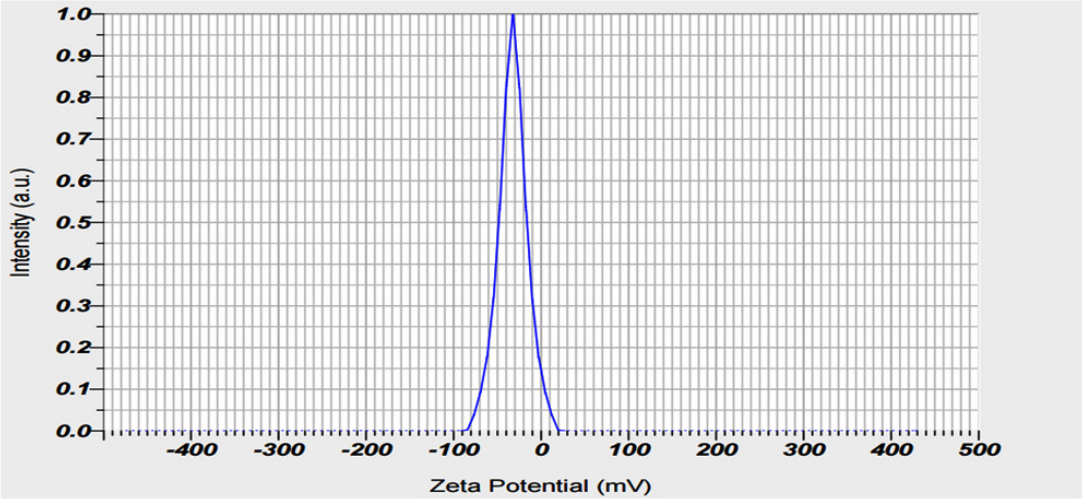

Fig. 5. NLCs Zeta graph (F4-Batch)

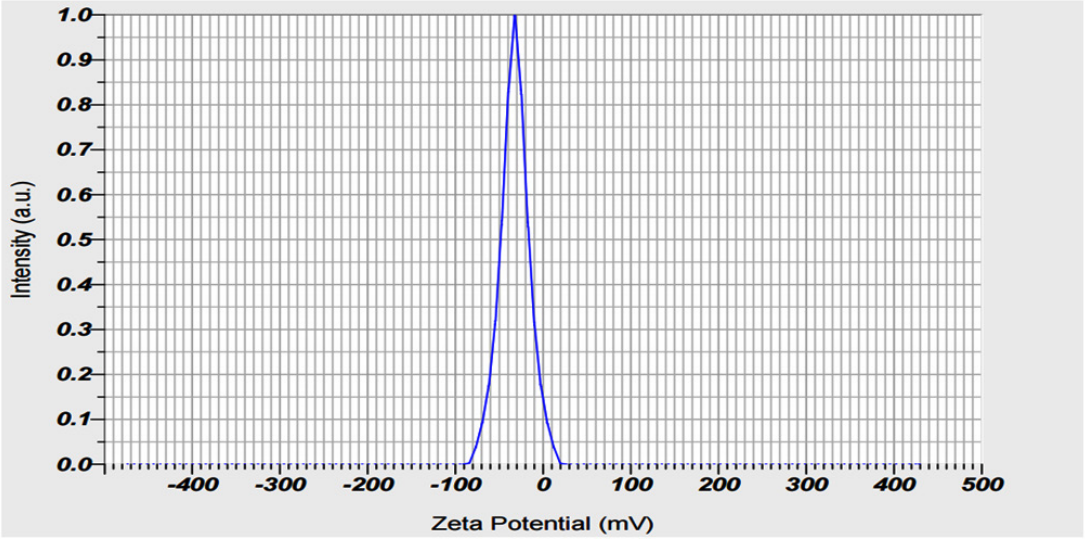

Fig. 6. NLCs loaded Thermosensitive gel Zeta graph (F4-Batch)

drug retention in the nasal passage. This ensures consistent therapeutic outcomes and reduces frequent dosing, optimizing nasal drug delivery.

Viscosity studies

The research assessed the viscosity profiles of six batches (F1 to F6) of thermosensitive gel formulations at two different temperatures, 4°C and 37°C. At 4°C, the viscosity values for all batches were relatively low, ranging from 17 to 28 centipoise (cp), indicating that the gels remain in a liquid state, which is ideal for ease of administration. Upon reaching body temperature (37°C), there was a significant increase in viscosity, with values ranging from 5026 to 5365 cp, reflecting the transition from a liquid to a gel state. This Thermoresponsive behavior ensures that the gel formulation can be easily applied intranasally at cooler temperatures and then forms a viscous gel at body temperature, enhancing drug retention and prolonged release within the nasal cavity.

Table 5. Table Cumulative Drug Release

Time (hours)	Cumulative drug release (%)		
	F 4 batch (NLC'S)	F 4 batch (TSG)	Pure drug release
2	7.23	8.053	20.65
4	12.565	14.729	45.25
6	19.565	20.071	60.25
8	27.69	29.006	82.63
10	38.20	41.845	98.23
12	45.23	50.916	
14	68.26	66.345	
16	70.56	73.710	
18	80.20	82.407	
20	86.23	89.503	
22	89.56	90.968	
24	91.25	92.682	

In-vitro drug release profile

The in-vitro drug release kinetics of the intranasal NLC loaded thermosensitive gel exhibit a detailed release profile marked by initial burst release phases. The total drug release percentage achieved during the investigation period is 92%, showcasing the effective encapsulation and subsequent release of the drug from the NLC platform.

The graph showed cumulative drug released (CDR) over 24 hours for three formulations: F4 Batch (NLC's), F4 Batch (TSG), and a marketed formulation. The marketed formulation released 50% CDR within 8 hours, reached nearly 90% by 24 hours. F4 Batch (TSG) and F4 Batch (NLC's) exhibited more gradual released, both achieving around 80% CDR by 24 hours. After 12 hours, both F4 Batch formulations maintained consistent release rates, indicating potential for sustained drug delivery. The results suggested that while the marketed formulation offered rapid initial release, F4 Batch formulations provided controlled, sustained release suitable for prolonged therapeutic used. The release kinetic follows zero order release.

Nasal permeation study

In vitro nasal permeation of Donepezil hydrochloride was specifically evaluated on Indian sheep (Deccani sheep) nasal mucosa. The NLCs-based thermosensitive gel demonstrated a 1.4-fold

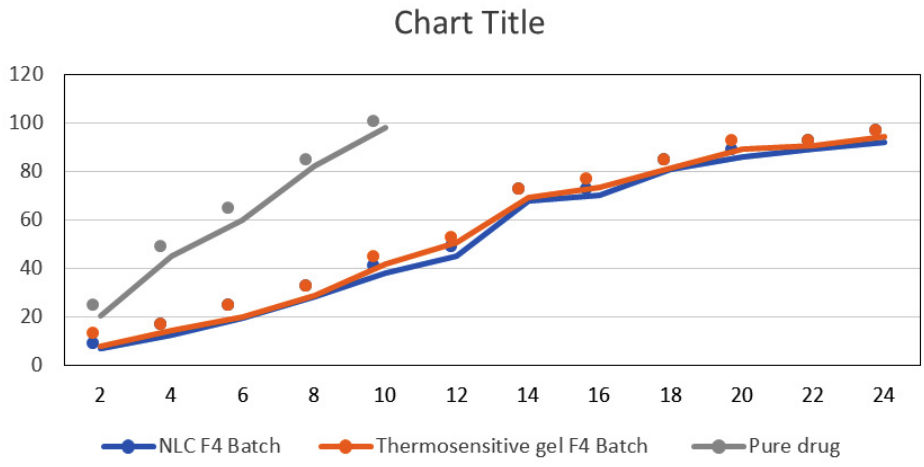

Fig. 7. Comparative study of % Cumulative drug release

rise in drug permeation compared to the plain DNP HCL gel. This enhanced permeation was attributed to the DNP HCL-loaded lipid nanoparticles in the NLCs, which provide an wide surface area for absorption into the highly vascularized nasal mucosa, leading to a higher concentration gradient for drug absorption. Additionally, surfactants in the NLCs helped to open the tight junctions in the nasal epithelial tissue, further enhancing drug permeation.

DISCUSSION

The development of nanostructured lipid carriers (NLCs) for intranasal delivery of donepezil hydrochloride demonstrated promising results for improving drug bioavailability and brain targeting. The NLCs prepared via the microemulsion technique exhibited stable characteristics, with particle size, zeta potential, and entrapment efficiency within desirable ranges for effective nasal administration. The integration of NLCs into a thermosensitive gel using Pluronic F127 further enhanced the formulation's properties, allowing for temperature-triggered gelation at physiological conditions (around 37°C). This thermoresponsive behavior facilitates easy administration and prolonged retention in the nasal cavity, which is advantageous for sustained drug release. In comparative drug release studies, the NLCs-loaded thermosensitive gel showed a controlled and sustained release profile compared to pure donepezil hydrochloride, highlighting the potential for reducing dosing frequency and improving therapeutic outcomes. The gel's ability to transition from a liquid to a semi-solid state upon reaching body temperature supports prolonged contact time with the nasal mucosa, enhancing drug absorption through the nasal epithelial pathways.

CONCLUSION

The research findings conclusively demonstrate the effectiveness of NLCs-based thermosensitive gel in improving drug delivery via nasal administration. The investigation indicates that the drug release process primarily adheres to zero-order kinetics, ensuring a consistent release rate regardless of drug concentration, which is advantageous for maintaining stable therapeutic levels. The nasal permeation analysis also underscores a substantial enhancement in drug permeation with the NLCs formulation compared to the plain drug gel, attributed to the increased surface area and surfactant activity that aid in drug absorption. These results suggest the potential of NLC-based thermosensitive gels as a superior drug delivery method for sustained and controlled release, with promising implications for treating conditions requiring prolonged drug administration. The formulation's reproducibility and reliability, coupled with its enhanced permeation and release characteristics, emphasize its suitability for further clinical development and implementation.

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor (Dr. Ganesh Shevalkar) for their invaluable guidance and support throughout this research. Special thanks to my family and friends for their encouragement and patience.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethical Sstatement

Sandip Institute of pharmaceutical sciences, Nashik

Authors contribution

Rohit S. Thete: Conceptualization, Methodology, Writing – Original Draft; Rohit S. Thete: Data Collection, Analysis, Writing – Review & Editing; Ganesh Shevalker and Laxmikant borse: Visualization, Supervision, Project Administration; Rohit S. Thete: Funding Acquisition, Resources, Supervision

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