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RESEARCH PAPER

Nose-to-Brain Delivery of Dolutegravir via Thermoresponsive Nanostructured Lipid Carriers: Cytocompatibility and Fluorescent Biodistribution Studies

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ABSTRACT

Delivering therapeutic agents to the brain remains a major challenge due to the restrictive nature of the blood–brain barrier (BBB). Intranasal administration has emerged as a promising, non-invasive approach that bypasses the BBB and facilitates direct nose-to-brain transport via the olfactory and trigeminal pathways. In this study, we developed a nanostructured lipid carrier (NLC) system for the intranasal delivery of dolutegravir sodium, a potent integrase inhibitor, with the goal of enhancing brain bioavailability for the treatment of neuroHIV and related central nervous system (CNS) complications. The NLCs were optimized for particle size, polydispersity index (PDI), and drug incorporation efficiency. The optimized formulation exhibited a mean particle size of 90.3 nm and a PDI of 0.23, indicating a uniform size distribution suitable for nasal administration. Cytocompatibility studies conducted on a model cell line confirmed the safety of the formulation. To enhance mucosal retention and enable sustained drug release, the NLC dispersion was incorporated into a thermosensitive in situ gel. Rhodamine B, a fluorescent dye, was used as a model tracer for qualitative assessment of biodistribution. In vivo studies in rats showed a time-dependent accumulation of rhodamine B in brain tissues following a single intranasal dose of the NLC in situ gel. Peak fluorescence was observed at 2 hours post-administration, confirming efficient brain targeting via the intranasal route. In conclusion, the developed in situ gel-based NLC system demonstrates potential as a non-invasive and effective platform for targeted brain delivery. This approach offers promising therapeutic opportunities for managing CNS disorders and warrants further investigation with clinically relevant drug molecules.

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INTRODUCTION

Human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND), collectively referred to as NeuroAIDS, represent a spectrum of neurological impairments ranging from mild cognitive decline to severe HIV-associated dementia. These conditions arise primarily due to HIV's ability to cross the blood–brain barrier (BBB) and establish reservoirs within the central nervous system (CNS), triggering

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chronic inflammation and neuronal injury that contribute to cognitive dysfunction [1,2]. Despite the success of combination antiretroviral therapy (cART) in controlling systemic infection, effective treatment of NeuroAIDS remains elusive because of limited drug penetration into the brain [3].

The BBB is a highly selective physiological barrier that restricts the entry of most antiretroviral drugs into the CNS, resulting in subtherapeutic drug levels that fail to suppress viral replication within brain tissues [4,5]. Various strategies to overcome the BBB, such as chemical modification of drugs, use of carrier-mediated transport, or invasive delivery techniques, have been investigated; however, each approach faces significant limitations including systemic toxicity, poor patient adherence, or inadequate CNS exposure [6]. Recent reviews underscore the urgent need for innovative delivery platforms that can safely and efficiently enhance drug bioavailability in the brain while minimizing off-target effects [7].

Intranasal administration has emerged as a promising non-invasive approach for direct nose-to-brain drug delivery, exploiting the unique anatomical pathways of the olfactory and trigeminal nerves that bypass the BBB [8]. This route offers distinct advantages, including rapid onset of action, reduced systemic exposure, and improved patient compliance. Nonetheless, challenges such as mucociliary clearance, enzymatic degradation, and limited nasal residence time continue to hamper therapeutic effectiveness [9,10]. Recent high-impact studies have demonstrated that formulation strategies enhancing nasal adhesion and sustained release can significantly improve brain targeting efficiency [11].

Nanostructured lipid carriers (NLCs) have garnered considerable attention as versatile nanocarriers for brain delivery due to their biocompatibility, high drug-loading capacity, and ability to protect therapeutic agents from enzymatic degradation [12–14]. Importantly, combining NLCs with thermosensitive mucoadhesive polymers to form in situ gels has shown promise in prolonging nasal residence time and enabling controlled drug release, thus addressing major limitations of intranasal delivery [15,16]. However, despite these advances, few studies have systematically optimized such composite systems for the delivery of antiretroviral agents targeting NeuroAIDS, highlighting a critical knowledge gap.

Dolutegravir sodium, a widely used integrase strand transfer inhibitor, has potent antiviral activity but limited CNS penetration due to poor BBB permeability when administered orally [17,18]. Although nanoparticle-based delivery systems for dolutegravir have been explored, they often neglect the nasal mucosal barrier or lack sustained release capabilities, underscoring the need for multifunctional formulations that enhance brain bioavailability via the intranasal route.

In selecting the literature referenced herein, priority was given to recent, high-impact primary studies and authoritative reviews published within the last decade that specifically address intranasal CNS delivery, lipid-based nanocarriers, and antiretroviral drug delivery challenges. This approach ensures a focused and credible background that reflects the current state of the art while identifying pertinent gaps.

This study aims to develop and characterize a thermoresponsive *in situ* gel formulation containing Rhodamine B-loaded nanostructured lipid carriers as a model system for evaluating nose-to-brain delivery efficiency. In parallel, a dolutegravir-loaded NLC in situ gel was formulated to assess cytocompatibility and potential therapeutic application in NeuroAIDS. By integrating nanocarrier technology with mucoadhesive thermosensitive gels, this work seeks to overcome both BBB and nasal mucosal barriers, providing a robust platform for sustained and targeted brain delivery of antiretroviral agents.

MATERIALS AND METHODS

Fluorescent dye (Rhodamine B) was sourced from Central Drug House.Glyceryl monostearate (GMS) and glycerol trioleate (triolein)were procured from Shanghai Macklin Biochemical Co. (China). Tween 40 and other analytical grade chemicals were acquired from Sisco Research Laboratories (India). Soluplus and Kolliphor® P 407 were supplied by D-BASF.

Preparation of Dolutegravir-Loaded Nanostructured Lipid Carriers (NLCs)

Dolutegravir-loaded NLCs were prepared using a modified melt emulsification–ultrasonication technique, a well-established method for lipid-based nanosystems. Briefly, predetermined amounts of solid lipid (glycerol monostearate) and liquid lipid (triolein) were selected based on prior solubility and compatibility screening. The

solid lipid was melted at approximately 5°C above its melting point to ensure complete liquefaction. Dolutegravir was then incorporated into the molten lipid phase at a concentration of 0.3% w/v. The mixture was continuously stirred using a magnetic stirrer to obtain a homogeneous lipid-drug melt.

Simultaneously, the aqueous phase was prepared by dissolving 4.0% w/v Tween® 40, a non-ionic surfactant with high emulsification capacity, and 50 mg Soluplus®, a polymeric solubilizer and stabilizer, in distilled water. The aqueous phase was heated to 80–85°C to match the temperature of the lipid phase, preventing premature solidification during mixing.

The hot aqueous phase was then added dropwise to the molten lipid-drug phase under continuous stirring at 850 rpm for 30 minutes, forming a coarse hot pre-emulsion.

To achieve nanoscale particle size with a narrow size distribution, the hot pre-emulsion was subjected to a two-step sonication protocol. Initially, the emulsion was treated in a temperature-controlled ultrasonic water bath maintained at 60 °C for 15 minutes to initiate preliminary droplet size reduction. This was followed by high-intensity probe sonication utilizing a titanium probe (diameter: 6 mm) operated at 30% amplitude in pulsed mode (2 seconds on, 2 seconds off) for a duration of 5 minutes. The sonication probe was consistently positioned 1.5 cm below the liquid surface, and batch volumes were standardized to 20 mL to ensure uniform acoustic energy distribution across formulations. To mitigate thermal degradation and maintain the lipid in a semi-molten state, the probe sonication step was conducted within an ice bath, thereby preventing the temperature from exceeding 60 °C. This dual sonication ensured efficient droplet size reduction and physical stability of the resulting NLC dispersion.

Preparation of Rhodamine B-Loaded NLCs

Rhodamine B-loaded NLCs were prepared following the same melt emulsification–ultrasonication procedure described above, substituting dolutegravir with Rhodamine B as the payload for biodistribution studies.

Preparation of NLC-Based In Situ Gel Formulation

The optimized Rhodamine B-loaded NLC dispersion was incorporated into an in situ gel composed of 19% Kolliphor® P 407 and 0.1% Carbopol to enhance nasal residence time and enable sustained release. The mixture was gently stirred until homogeneous and stored at 4 °C until further use.

At a Poloxamer 407 (Kolliphor® P 407) concentration of 19% w/v and 0.1% Carbopol,the formulation exhibited an optimal gelation temperature of approximately 34 °C, closely matching physiological nasal mucosa temperature. This thermoresponsive behavior ensures the gel remains fluid during administration but rapidly gels upon contact with the nasal cavity, promoting prolonged mucosal residence and sustained drug release.

Characterization of NLCs

The particle size (PS) and polydispersity index (PDI) of the NLC formulations were measured using dynamic light scattering (DLS) with a Malvern Zetasizer Ultra (Malvern Instruments, UK). Prior to analysis, samples were diluted 1:50 with double distilled water (DW) to avoid multiple scattering effects while maintaining an adequate scattering intensity. The dilution ratio was kept consistent across all samples to ensure comparability.

Measurements were performed at 25 °C, following an equilibration time of 120 seconds. The refractive index for the dispersed phase was set to 1.59, corresponding to polystyrene latex standards used for instrument calibration, while the refractive index of the dispersant (water) was set at 1.33. The viscosity of the dispersant was considered as 0.8872 cP.

All samples were analyzed in triplicate, and data were reported as the mean ± standard deviation. The instrument's default settings for the number of runs per measurement and data analysis model (general purpose mode with non-negatively constrained least squares fit) were used unless otherwise stated.

Determination of Encapsulation Efficiency

The encapsulation efficiency (EE%) of the Dolutegravir-loaded NLCs were quantified using an indirect ultrafiltration method followed by UV–Visible spectrophotometric analysis.

Briefly, 2mL of the NLC dispersion was placed into a preconditioned ultrafiltration centrifuge tube (Amicon® Ultra-4, 10 kDa molecular weight cut-off) and centrifuged at 4000 rpm for 20 minutes at 4 °C using a refrigerated centrifuge

(Eppendorf 5430 R). The filtrate containing unencapsulated (free) drug was collected, and the concentration of Dolutegravir was determined spectrophotometrically at λ_max = 259 nm using a validated UV–Vis method (Shimadzu UV-1900, Japan).

Entrapment efficiency = (Total amount of drug added-Amount of drug in superntant)/(Total amount of drug)

MTT Cell Viability Assay

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay was utilized to assess cell viability of HFF and metabolic activity after treatment with DTG-NLC. To prepare the MTT stock solution, 5 mg of MTT powder (Sigma-Aldrich, USA) was precisely weighed and dissolved in 1 mL of sterile phosphate-buffered saline (PBS, pH 7.4). The solution was then sterilized by filtration through a 0.22 µm syringe filter to eliminate any particulate matter. Aliquots of the filtered solution were stored in microcentrifuge tubes, protected from light with aluminum foil, and kept at −20°C until required.

Cells were plated in 96-well plates at a density of 1×10^4 cells per well in 100 µL of complete culture medium. The plates were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 24 hours to allow for proper cell attachment and stabilization. After this period, the cells were exposed to various experimental treatments for the specified durations.

Following treatment, the MTT assay was conducted to determine cell viability. In brief, 100 µL of the prepared MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for 3–4 hours at 37°C. During this time, metabolically active cells reduced the yellow tetrazolium salt into insoluble purple formazan crystals. After incubation, the MTT-containing medium was gently removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The plates were further incubated at 37°C for 15 minutes to ensure complete solubilization. Finally, absorbance was measured at 570 nm using a microplate spectrophotometer [18].

Cell viability was expressed as a percentage of the control (untreated) group using the following formula:

Cell viability (%) = (Absorbance of control sample/Absorbance of treated sample) × 100

Animal study design

All animal procedures were conducted in accordance with the ethical standards approved by the Research Ethics Committee for Experimental Investigations at the College of Pharmacy, University of Baghdad, Iraq (Approval No. [REC02202507A.]). The study adhered to the national guidelines for the care and use of laboratory animals.

Animal Housing and Care

Animals were housed in standard polypropylene cages (2 rats per cage) under controlled conditions: temperature 22 ± 2 °C, relative humidity 55 ± 10%, and a 12:12 h light/dark cycle. They were given free access to standard pellet chow and water ad libitum. All animals were acclimatized for 7 days before initiating the experimental protocol to minimize stress-related variability.

To assess the brain-targeting efficiency of the Rhodamine B-loaded nanostructured lipid carrier (NLC) in situ gel, ten adult female Wistar rats (weighing 190–220 g) were randomly assigned to two groups using a computer-generated randomization list: a control group (n = 2), which received no treatment, and a treatment group (n = 8), which received a single intranasal administration of 40 µL Rhodamine B-loaded NLC in situ gel via a calibrated micropipette. The 40 µL dose volume was selected based on previously published intranasal delivery studies in rodents, representing the upper limit of nasal cavity capacity and tolerability for a single administration.

The sample size reflects established precedents in pilot biodistribution studies, wherein 2 animals per time point are routinely employed to evaluate preliminary brain-targeting patterns using qualitative imaging techniques such as fluorescence microscopy. Although formal power calculations were not conducted due to the exploratory and qualitative nature of the endpoints, this sample size was considered adequate for proof-of-concept and temporal biodistribution assessment. Future studies will include statistical power analyses informed by these preliminary findings to ensure robust quantitative evaluation.

At predetermined time points (0, 0.5, 1, 2, and 5 hours post-administration), two animals were sacrificed at each interval. Euthanasia was performed via diethyl ether overdose followed by cervical dislocation, a method approved within the institutional protocol at the time of the

study. Although it is acknowledged that diethyl ether is not considered a first-line method in current international ethical guidelines due to variability and potential animal distress, its use was constrained by institutional availability and regulatory acceptance. Strict precautions were taken to ensure operator safety and minimize animal discomfort, and all procedures were conducted in a certified fume hood.

Fluorescence Imaging and Image Analysis

The fluorescence imaging was performed using a Zeiss fluorescence microscope (Zeiss, Germany) equipped with rhodamine-specific filters (excitation range: 530–570 nm). Images were captured using a Zeiss Axiocam 202 mono digital camera, with standardized exposure times and acquisition settings applied across all samples to ensure consistency. Background fluorescence was measured from regions lacking specific signal and uniformly subtracted. Image contrast was adjusted in Adobe Photoshop solely to enhance visibility while preserving the integrity and accuracy of the data.

Stability study

Stability studies were conducted to assess the physicochemical integrity of the Dolutegravir Sodium-loaded nanostructured lipid carrier (DTG-NLC) in situ gel formulation over time. The optimized formulation was stored in airtight, light-protective glass containers under two storage conditions: refrigeration at 4 °C and ambient room temperature at 25 °C, for a duration of three months. At predetermined intervals (0, 1, 2, and 3 months), samples were withdrawn and subjected to systematic evaluation.Preliminary visual inspection was performed to detect any signs of physical instability, such as precipitation, phase separation, or color change. Subsequently, the samples underwent comprehensive physicochemical analysis, including measurement of particle size (PS), polydispersity index (PDI), pH, entrapment efficiency (EE%), and drug content. These parameters were selected to monitor potential alterations in formulation performance and structural integrity over time. The study aimed to confirm the formulation's stability and long-term suitability for intranasal delivery and therapeutic application in CNS-targeted drug delivery.

RESULTS AND DISCUSSION

Physicochemical Characterization of DTG-Loaded NLCs

The drug-loaded nanostructured lipid carriers (NLCs) exhibited a mean particle size of 90.33 nm and a polydispersity index (PDI) of 0.23, indicating a narrow and homogeneous size distribution (Fig. 1). The encapsulation efficiency was found to be 80.1%, reflecting effective drug incorporation within the lipid matrix. The physicochemical characteristics of the optimized DTG-loaded NLCs strongly support their potential for intranasal drug delivery to the brain. The mean particle size of 90.33 nm and low polydispersity index (PDI = 0.23) fall within the ideal range for efficient nose-to-brain transport. Nanoparticles below

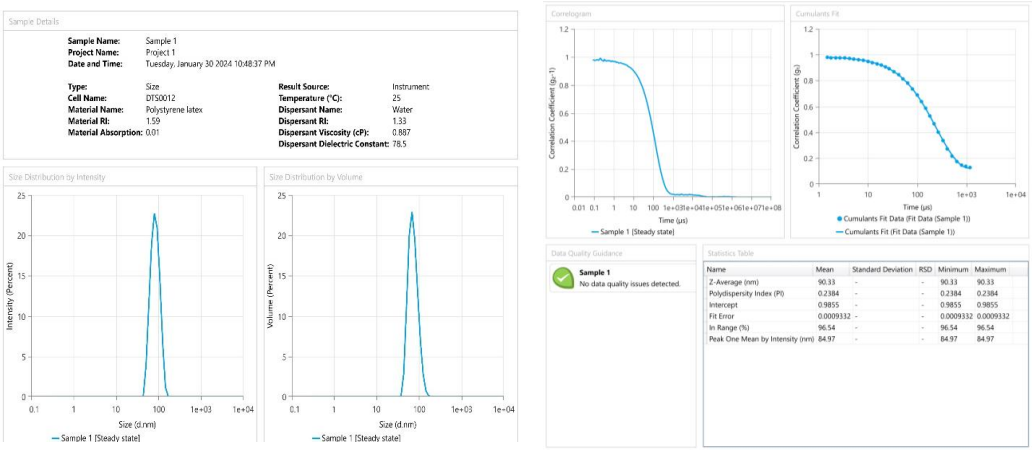

Fig. 1. particle size distribution of DTGs-NLC using malvern zetasizer.

100 nm are widely recognized to have superior permeation across the nasal epithelium and enhanced uptake via the olfactory and trigeminal pathways, facilitating direct access to the central nervous system (CNS) without relying on systemic circulation [19-22]. The narrow size distribution ensures consistent absorption and predictable pharmacokinetics, both of which are crucial for therapeutic effectiveness. A PDI of less than 0.3 is typically favored for intranasal formulations, as lower PDI values, along with smaller particle sizes, promote uniform drug absorption across the nasal mucosa [23]. Furthermore, the narrow size distribution indicated by the PDI of 0.23 results in consistent mucosal retention, reliable uptake, and a higher degree of reproducibility between batches, all of which are essential for achieving therapeutic efficacy in brain targeting applications.

The high encapsulation efficiency (EE) of 80.1% observed for nanostructured lipid carriers (NLCs)

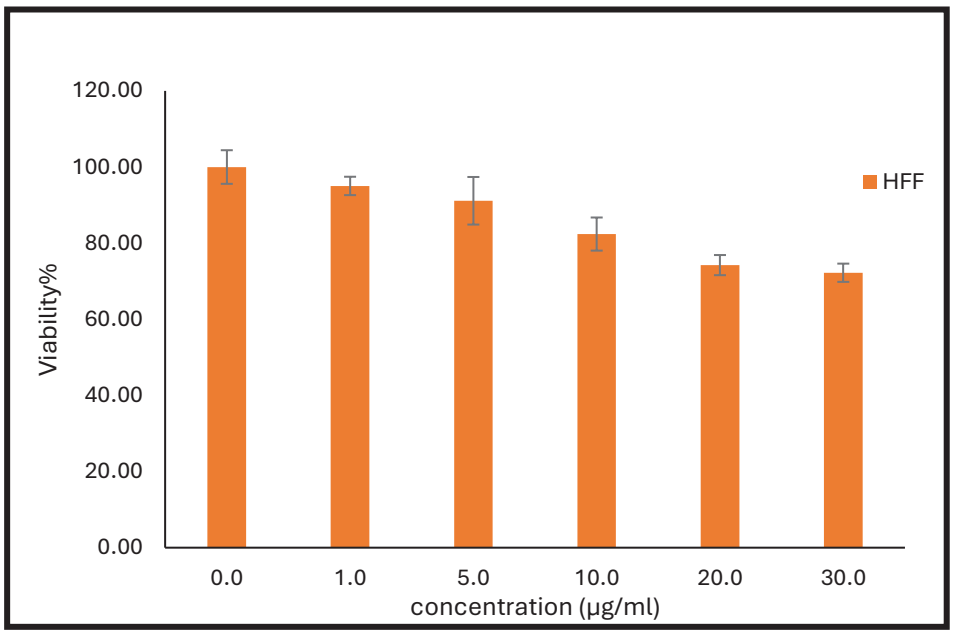

Fig. 2. % Cell viability at different concentrations.

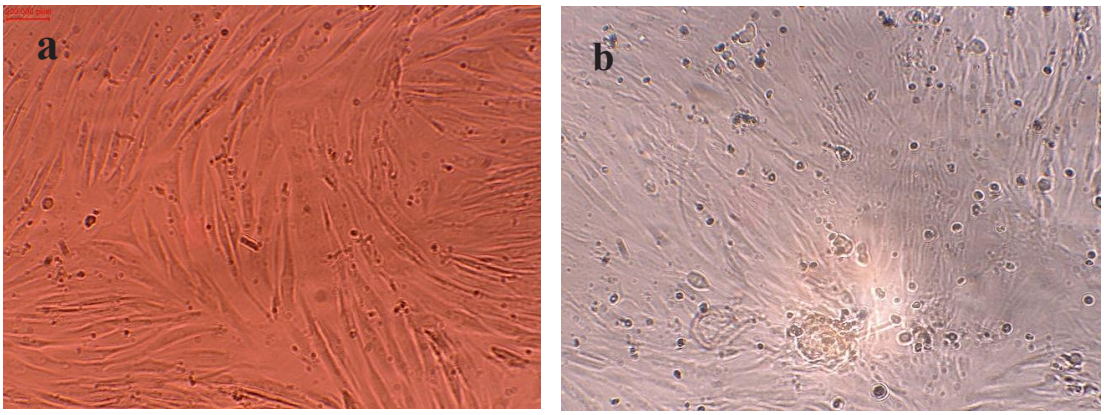

Fig. 3. Cell viability was assessed using the MTT assay in human foreskin fibroblast (HFF) cells, comparing (a) untreated controls and (b) cells exposed to the DTGs-loaded nanostructured lipid carriers (DTGs-NLC.)

is a critical indicator of successful drug entrapment within the lipid matrix, which has direct implications for therapeutic performance. High EE reflects the carrier's ability to accommodate and securely incorporate active pharmaceutical ingredients, particularly those with poor water solubility, within its hydrophobic core. This robust encapsulation not only minimizes premature drug release during storage and transit through physiological environments but also contributes to maintaining a consistent and controlled drug release profile at the target site. [24-25]. In the context of nose-to-brain delivery, an EE of 80.1% ensures that a substantial amount of the drug is delivered through the olfactory or trigeminal pathways, maximizing payload arrival at the central nervous system and overcoming limitations associated with conventional delivery routes.

In Vitro Cytocompatibility

Cell viability studies demonstrated excellent cytocompatibility of the formulation. No morphological signs of cellular damage or detachment were observed under microscopy (Fig. 2). Quantitative MTT assay revealed a survival rate of 95.04 ± 4.83% at 1 µg/mL, which declined to 72.22 ± 4.8% at 30 µg/mL (Fig. 3). The IC_{50} value was determined to be 50.41 µg/mL against human foreskin fibroblast (HFF) cells. The *in vitro* cytocompatibility results provide further evidence of the safety profile of the NLC formulation. Cell viability remained above 70% even at the highest tested concentration, and the IC_{50} value of 50.41 µg/mL suggests that the formulation is only moderately cytotoxic at elevated doses. This safety margin is reassuring for intranasal use, where direct contact with mucosal tissues could otherwise raise toxicity concerns. The high cell viability aligns with the inherent advantages of NLCs, which are composed of biocompatible lipids (e.g., glycerol monostearate, triolein) and surfactants (e.g., Tweens, Poloxamer 188). These components are generally regarded as safe (GRAS) by regulatory agencies like the FDA, contributing to the low cytotoxicity observed [26]. The lipid matrix of NLCs, unlike purely synthetic nanoparticles (e.g., polymeric nanoparticles), mimics biological membranes, reducing the likelihood of cellular damage [27].

This is particularly important for nose-to-brain delivery, where the formulation must avoid toxicity to the nasal mucosa and olfactory/trigeminal nerve endings to ensure safe passage to the central nervous system (CNS). Several studies corroborate the low cytotoxicity and high biocompatibility of NLCs for intranasal delivery, supporting the findings you provided [28].

In Vivo Biodistribution

The biodistribution study using Rhodamine B-loaded NLC in situ gel showed time-dependent brain accumulation. Weak fluorescence signals were detected at 30 and 60 minutes post-administration, with prominent signal localization in the olfactory bulb. Maximum fluorescence intensity was observed at 2 hours, followed by a gradual decline by 5 hours, although signal remained detectable (Fig. 4). *In vivo* biodistribution studies using Rhodamine B as a fluorescent probe confirmed successful and time-dependent delivery of the compound to brain tissues following intranasal administration.

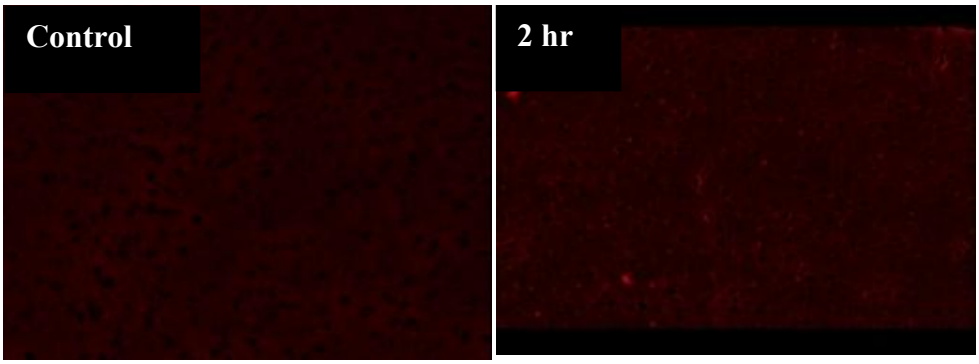

Fig. 4. Fluorescence images of brain tissue from the control group, which received no treatment, and from the groups administered Rhodamine B-loaded nanostructured lipid carrier (NLC) in situ gel via the intranasal route.

The use of Rhodamine B as a fluorescent probe is a well-established approach for tracking nanoparticle biodistribution due to its lipophilic nature and fluorescence properties, which mimic the behavior of hydrophobic drugs encapsulated in NLCs.

The rapid detection of Rhodamine B fluorescence in the olfactory bulb following intranasal administration highlights the efficiency of NLCs in exploiting the olfactory pathway for direct brain delivery. The olfactory bulb, located in close proximity to the nasal cavity, serves as a primary entry point for intranasally administered compounds, bypassing the BBB. The observed peak fluorescence intensity at 2 hours suggests that NLCs facilitate swift transport, likely via transcellular or paracellular mechanisms through olfactory epithelial cells and subsequent axonal transport along olfactory neurons. This rapid onset is consistent with the anatomical advantages of the nose-to-brain route, where the olfactory mucosa provides direct access to the CNS, avoiding systemic circulation and first-pass metabolism [29].

The retention of fluorescence in the brain up to 5 hours post-administration indicates prolonged residence and sustained release of the encapsulated compound, a hallmark of NLCs' ability to provide controlled drug delivery. Unlike solid lipid nanoparticles (SLNs), which may exhibit limited drug loading and release profiles, NLCs incorporate a blend of solid and liquid lipids, creating an imperfect matrix that enhances drug solubility and sustains release. This prolonged residence is critical for therapeutic applications, as it ensures a steady drug concentration in the CNS, potentially improving efficacy for conditions like Alzheimer's disease, Parkinson's disease, or brain tumors, where sustained drug action is required [30-31].

Stability Study

As shown in Fig. 5, the optimized formulation remained stable over three months. No statistically significant changes were noted in particle size, entrapment efficiency, or pH across storage temperatures. Minor variations were observed at 25 °C, but all values remained within acceptable

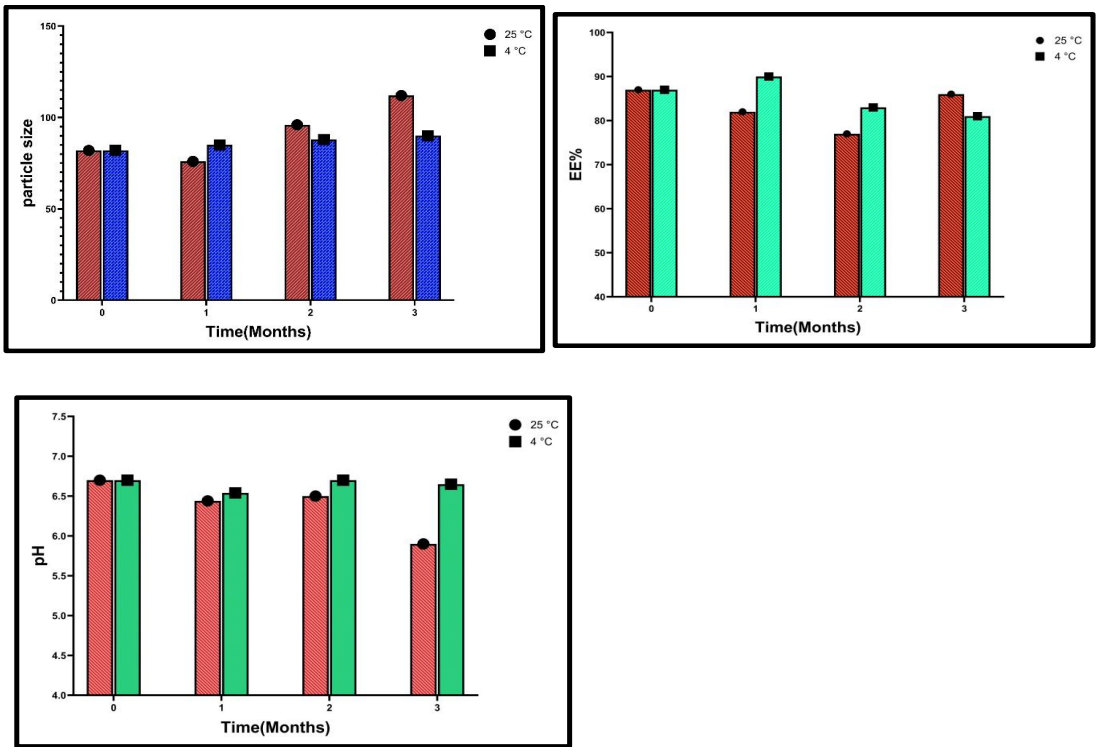

Fig. 5. Stability parameters (particle size, Entrapment efficiency, pH of the optimized nanoformulation during storage at various temperatures.

limits. the stability study demonstrated that the NLC-based in situ gel maintained its structural and functional integrity over a three-month period, with no significant changes in key parameters. This level of stability is vital for practical formulation development and clinical translation.

Collectively, these findings provide compelling evidence that the DTG-loaded NLC-based in situ gel represents a promising, non-invasive strategy for targeted nose-to-brain delivery of antiretroviral drugs. However, while the preclinical results are encouraging, further investigations using therapeutic doses of Dolutegravir Sodium, detailed pharmacokinetic profiling, and efficacy studies in relevant disease models are necessary to fully validate the clinical applicability of this platform in the treatment of neuroHIV and other CNS disorders.

CONCLUSION

This study successfully developed and characterized a nanostructured lipid carrier (NLC)-based thermosensitive in situ gel for the intranasal delivery of Dolutegravir Sodium, aimed at improving brain targeting for the potential management of neuroHIV and associated CNS complications. The optimized NLC formulation demonstrated favorable physicochemical properties, including a uniform particle size and high encapsulation efficiency, and showed good cytocompatibility in vitro. Incorporation into an in situ gel enhanced mucosal retention and supported sustained release behavior. Preliminary in vivo biodistribution using Rhodamine B as a model fluorescent probe suggested efficient, time-dependent brain accumulation following intranasal administration. However, these results are based on a model compound and limited in vivo assessments. Therefore, while the findings are promising, further investigations involving therapeutic drug loading, comprehensive pharmacokinetic and pharmacodynamic studies, and long-term safety evaluations are essential to fully validate the clinical potential of this delivery platform for effective and non-invasive nose-to-brain drug transport.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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