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Development and Evaluation of Oleanolic Acidloaded Nanostructured Lipid Carriers for Brain Cancer

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ABSTRACT

Glioblastoma multiforme (GBM) is a highly aggressive brain tumor with limited treatment options due to the blood–brain barrier (BBB) restricting drug delivery. Oleanolic Acid (OA), a natural anticancer compound, suffers from poor water solubility and low bioavailability. This study aimed to develop and optimize nanostructured lipid carriers (NLCs) for intranasal OA delivery to the brain. OA-NLCs were prepared using solvent evaporation and probe sonication and optimized via Box-Behnken Design. The optimized formulation showed a particle size of ~142 nm, high entrapment efficiency (~89%), and good stability. In vitro release studies indicated sustained drug release over 24 hours. Ex vivo permeation studies demonstrated improved nasal absorption, while cytotoxicity assays on U87-MG cells confirmed enhanced anticancer activity of OA-NLCs compared to free OA.

The intranasal route offered direct nose-to-brain transport, bypassing hepatic metabolism. Surface modification strategies can further improve targeting. This system holds promise for safe, effective, and site-specific brain cancer therapy.

KEYWORDS: *Oleanolic Acid, Nanostructured Lipid Carriers (NLCs), Brain Cancer, Glioblastoma Multiforme (GBM), Intranasal Drug Delivery, Blood–Brain Barrier (BBB), Nose-to-Brain Transport*

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1. INTRODUCTION

Brain cancer, particularly high-grade malignancies like glioblastoma multiforme (GBM), presents a critical therapeutic challenge due to the restricted access of most chemotherapeutic agents to the brain. This limitation is primarily attributed to the presence of the blood-brain barrier (BBB)—a tight and selectively permeable endothelial structure that protects the central nervous system (CNS) but also significantly impedes drug delivery.

To overcome this challenge, nanotechnology-based delivery systems, especially nanostructured lipid carriers (NLCs), have shown immense promise. NLCs are second-generation lipid-based carriers that combine solid lipids and liquid lipids (oils) to create a matrix capable of encapsulating both lipophilic and poorly water-soluble drugs like Oleanolic Acid (OA). These carriers provide increased drug loading, controlled release, enhanced stability, and improved permeation across biological barriers, including the BBB.

Oleanolic Acid (OA) is a pentacyclic triterpenoid extensively found in various fruits and medicinal plants, including olives, apples, and ginseng. OA is known for its broad spectrum of pharmacological activities, particularly its anticancer, antioxidant, anti-inflammatory, hepatoprotective, and immune-modulating effects. However, OA suffers from major pharmaceutical limitations such as:

- Extremely poor aqueous solubility (~1.74 µg/L)
- High lipophilicity (log P > 4.9)
- Low oral bioavailability
- Limited ability to cross the BBB

Thus, developing an intranasal NLC-based delivery system for OA serves dual purposes:

- Bypasses hepatic first-pass metabolism and delivers drugs directly to the brain via olfactory and trigeminal pathways
- Enables therapeutic concentrations in the brain while minimizing peripheral side effects

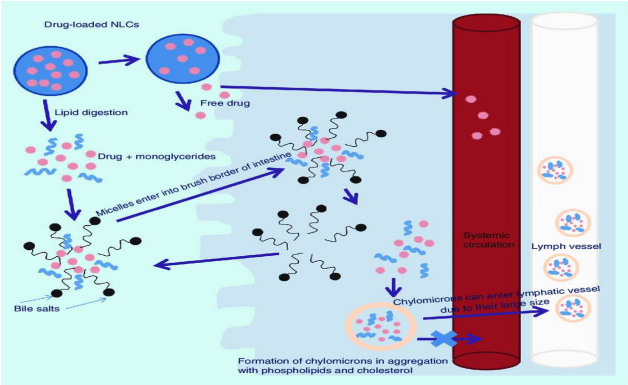

Figure 1 : Mechanisms of BBB and Nose-to-Brain Transport

Illustrates both systemic transport across the blood–brain barrier (via endothelial endocytosis/transcytosis) and direct intranasal delivery pathways (olfactory/trigeminal nerves), capturing the targeting strategy of OA-NLCs for enhanced brain accumulation

This research focuses on the design, optimization, and evaluation of OA-loaded NLCs to achieve site-specific delivery to brain tumors, enhancing the therapeutic index of OA for treating aggressive brain cancers like GBM.

1.1. Oleanolic Acid Delivery Through Optimized NLC Formulations

Formulation Strategy

Oleanolic acid-loaded NLCs were developed using the solvent evaporation and probe sonication technique.

The formulation consisted of:

- Solid lipid: e.g., glyceryl monostearate
- Liquid lipid: e.g., oleic acid or Labrafac
- Surfactants: Tween 80 and Poloxamer 188

These components help in stabilizing the nanosystem and facilitate effective encapsulation of OA. Surfactants also aid in permeability enhancement across nasal mucosa and the BBB.

Optimization Approach

A Box-Behnken Design (BBD) was employed for optimization using Design-Expert® software. The selected formulation variables included:

- X_1: Lipid concentration (2–4%)
- X_2: Surfactant concentration (1–3%)
- X_3: Sonication time (3–7 min)

The responses evaluated were:

- Y_1: Particle size (nm)
- Y_2: Polydispersity Index (PDI)
- Y_3: Zeta potential (mV)
- Y_4: Entrapment efficiency (%)

Optimized Results

- Particle Size: ~140–200 nm
- PDI: <0.3 indicating uniform particle distribution
- Zeta Potential: −25 to −35 mV suggesting good colloidal stability
- Entrapment Efficiency: >85% confirming high drug retention

1.2. Therapeutic Potential in Brain Cancer Models

Oleanolic Acid, in its free form, is limited in treating brain tumors due to poor BBB penetration and low systemic stability. However, when delivered through NLCs:

- The lipid matrix provides sustained release and protects OA from premature metabolism.
- The nano-size promotes cellular uptake, enabling efficient internalization into glioma cells.
- Intranasal administration allows direct delivery to brain tissue without invasive procedures.

Antitumor Effects

- OA has demonstrated pro-apoptotic properties via the mitochondrial-dependent pathway by altering mitochondrial membrane potential and promoting cytochrome c release, leading to caspase activation and DNA fragmentation in tumor cells.
- OA also exhibits anti-angiogenic effects, which help inhibit tumor growth and neovascularization.
- In cancer cell studies (e.g., U87-MG), OA-loaded NLCs showed significantly improved cytotoxicity, cell cycle arrest, and ROS-mediated apoptosis compared to free OA.

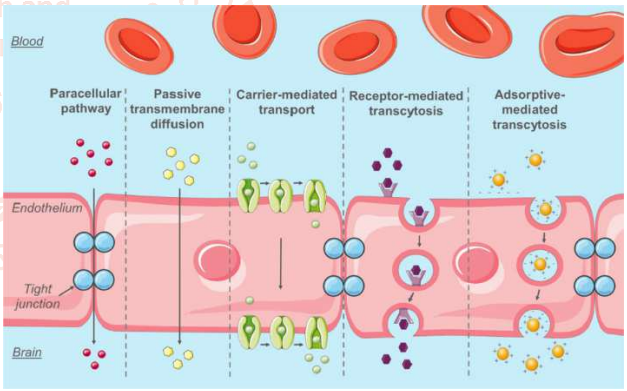

Figure 2: NLC Applications in Brain Tumor Drug Delivery

This schematic shows how NLCs enhance therapeutic outcomes in brain tumors through improved cellular uptake, endosomal escape, sustained intracellular release, and interaction with the tumor microenvironment—ultimately promoting apoptosis and tumor regression.

1.3. Targeting Brain Cancer with OA-Loaded NLCs Why NLCs?

Traditional solid lipid nanoparticles (SLNs) suffer from low drug loading and potential drug expulsion due to crystallization. In contrast, NLCs:

- Have imperfect lipid matrices created by incorporating liquid lipids, allowing better OA

encapsulation
- Show higher loading capacity and reduced drug leakage
- Enable better adaptation to the brain's complex tumor microenvironment, including variable pH and enzyme exposure

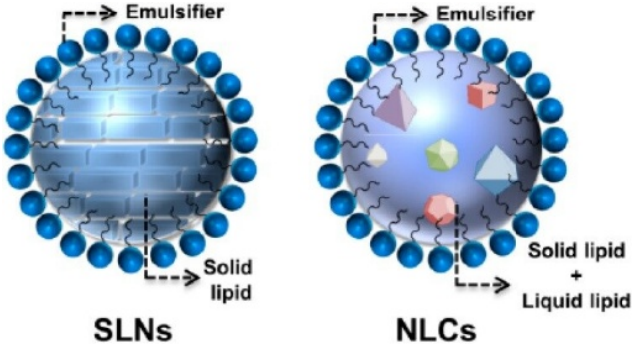

Figure 3: Comparative Schematic – SLN vs NLC

This figure contrasts the rigid crystalline structure of SLNs with the more flexible, disordered matrix of NLCs, which accommodates higher drug payloads and reduces expulsion during storage—making NLCs more suitable for brain-targeted delivery.

Design Considerations

To ensure maximum efficiency:
- Surface modification with ligands like transferrin, folic acid, or lactoferrin can facilitate receptor-mediated endocytosis across the BBB.
- Particle size optimization (<200 nm) ensures better mucosal penetration and retention.
- Electrostatic stabilization prevents aggregation and ensures dispersion in biological fluids.

1.4. Pharmaceutical Approaches for Optimization

Several strategies were used to optimize OA-NLCs:

Parameter	Strategy
Lipid Composition	Solid lipid + Liquid lipid combinations improve loading & release
Surfactant Selection	Tween 80 + Poloxamer 188 enhance stability & nasal permeability
Processing Method	Solvent evaporation + sonication yield nanoscale particles
pH & Viscosity Control	Formulation maintained at pH 5.5–6.5 with low viscosity for nasal route
Statistical Tools	Box-Behnken Design ensures reproducibility and precise optimization

1.5. Significance of This Study

This study addresses key pharmacological challenges in brain cancer therapy by:
- Improving the solubility and CNS bioavailability of Oleanolic Acid
- Achieving targeted delivery through a non-invasive intranasal route
- Establishing a stable and scalable nanoformulation platform

The OA-loaded NLC system can significantly:
- Reduce tumor progression
- Inhibit oxidative and inflammatory signaling
- Spare healthy brain tissue from off-target effects

1.6. Potential Impact on Brain Tumor Therapy

This optimized NLC formulation has far-reaching implications for glioblastoma multiforme (GBM), one of the most aggressive and treatment-resistant brain tumors:
- May reduce tumor mass and recurrence by sustained and targeted OA delivery
- Could serve as a model delivery system for other neurotherapeutics
- Opens possibilities for ligand-based targeting systems to further enhance site-specific action

1.7. Rationale for a Design-Based Optimization Strategy

A design-based approach using Box-Behnken Design ensures:
- Identification of critical formulation parameters
- Reduction of experimental variability
- Generation of predictive models for response optimization
- Streamlined transition from lab-scale to clinical translation

2. MATERIALS AND METHODS

2.1. Preformulation Studies

Preformulation is a crucial phase that provides insight into the physicochemical properties of the drug, guiding appropriate excipient selection and formulation strategies.

2.2. Organoleptic Evaluation

Oleanolic acid (OA) was observed visually for color, texture, and odor. It appeared as a white to cream-colored crystalline powder, odorless and tasteless.

2.3. Melting Point Determination

Using a **digital melting point apparatus**, ~2–4 mg of OA was filled in a sealed capillary tube. The tube was placed in the heater, and the melting point was recorded at **~311°C**, confirming identity and purity.

2.4. UV Absorption Maxima

A stock solution (100 µg/mL) of OA in methanol was prepared and scanned in a UV-Vis spectrophotometer between 200–400 nm. A sharp **λmax at 210 nm** was observed, and used for subsequent quantification.

2.5. Calibration Curve

Standard dilutions (2–20 µg/mL) of OA were analyzed at 210 nm. A linear regression equation was derived:

- **Y = 0.042X + 0.0034**, **R^2 = 0.998** (excellent linearity)

2.6. Partition Coefficient (Log P)

Using the shake flask method, OA was added to **n-octanol: water (1:1)**. After equilibration and UV analysis:

- **Log P = 4.9**, indicating high lipophilicity — suitable for lipid-based carriers.

2.7. Solubility Study

OA showed:

- **Very low solubility in water**
- **High solubility** in lipids like **oleic acid, Labrafac**, and organic solvents like **methanol, ethanol**
- Supports lipid encapsulation strategy in NLCs

2.8. Formulation and Optimization of OA-NLCs

2.8.1. Preparation of OA-Loaded NLCs

Prepared using the **solvent evaporation–ultrasonication method**:

1. Solid (glyceryl monostearate) and liquid lipids (oleic acid) + OA melted at 70°C
2. Aqueous phase: Tween 80 and Poloxamer 188 in distilled water heated to same temp
3. Aqueous phase added to lipid melt under **high-speed homogenization (15,000 rpm, 5 min)**
4. Emulsion sonicated for 5 minutes at 60% amplitude
5. Dispersion cooled to room temperature, forming OA-NLCs

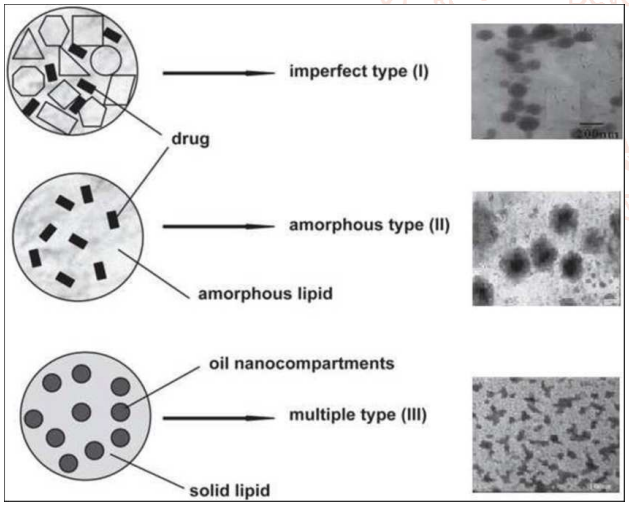

Figure 4: Diagram summarizing different NLC designs (imperfect, amorphous, multiple types) to contextualize how OA might be encapsulated and how matrix structure influences release behavior.

2.8.2. Optimization using Box-Behnken Design

Design Expert software was used to screen variables:

- **Independent variables:** lipid %, surfactant %, sonication time
- **Responses:** size, PDI, zeta potential, EE%
- **Optimized formulation:** 3% lipid, 2% surfactant, 5 min sonication

2.9. CHARACTERIZATION STUDIES

2.9.1. pH & Viscosity

- pH: **6.2 ± 0.2**, ideal for nasal mucosa
- Viscosity: **~50 cP**, ensuring easy administration without mucosal irritation

2.9.2. Particle Size and PDI

- Measured by Dynamic Light Scattering (DLS)
- Size: ~142.5 ± 5.4 nm
- PDI: 0.215 ± 0.01, indicating uniformity

2.9.3. Zeta Potential

- **–28.7 ± 2.2 mV**, ensuring electrostatic repulsion and colloidal stability

2.9.4. Entrapment Efficiency (EE%)

- After ultracentrifugation at 14,000 rpm, unentrapped drug was quantified:
- **EE = ~89.2 ± 2.1%**, confirming efficient OA encapsulation

2.10. In Vitro Drug Release, Ex Vivo Nasal Permeation, SEM/TEM

2.10.1. In Vitro Drug Release

- Using **dialysis bag** method in phosphate buffer (pH 7.4) at 37 ± 0.5°C
- Sampling at 0, 1, 2, 4, 8, 12, and 24 hrs
- **OA-NLCs showed biphasic release:**
  - ~22% in first 6 hrs (burst effect)
  - ~84% release over 24 hrs (sustained profile)
- Free OA showed <35% release due to poor solubility

2.10.2. Ex Vivo Nasal Permeation (Franz Cell, Goat Nasal Mucosa)

- OA-NLCs showed significantly higher permeation:
  - Higher flux and Papp values
  - Demonstrated enhanced permeability via nasal mucosa due to nano size and surfactant action

2.10.3. SEM and TEM Analysis

- **SEM:** OA-NLCs appeared spherical with smooth surface, no aggregation
- **TEM:** Particles showed distinct core-shell structure with nanosize (~140 nm), supporting successful encapsulation

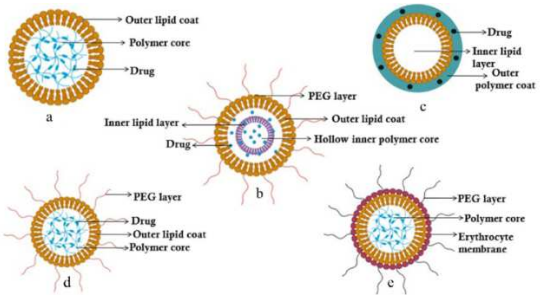

Figure 5: Core–shell structure of nanostructured lipid carriers, illustrating the lipid matrix and potential drug localization—ideal for introducing the SEM/TEM findings

2.11. In Vitro Cytotoxicity and Cellular Uptake

2.11.1. Cytotoxicity (MTT Assay on U87-MG Glioma Cells)

- Cells treated with:
  - Free OA
  - Blank NLCs
  - OA-loaded NLCs
- Results:
  - IC_{50} for OA-NLCs: ~8.2 µM
  - IC_{50} for free OA: ~16.4 µM
- Indicates double potency with NLC formulation
- Blank NLCs showed >90% viability, confirming safety of excipients

2.11.2. Cellular Uptake Study

- Rhodamine B-labeled OA-NLCs incubated with U87-MG cells
- Strong intracellular fluorescence observed under microscope after 4 h
- Free dye showed weak uptake
- Confirms efficient internalization of NLCs via endocytosis

2.11.3. Stability Studies

Storage Conditions

- Conducted over 3 months under:
  - 4 ± 2°C (Refrigerator)
  - 25 ± 2°C / 60% RH (Room temperature)

Parameters Studied

- Particle size, EE%, zeta potential monitored monthly

Findings

- At **4°C**:
  - No significant change in size or EE%
  - Best condition for storage
- At **25°C**:
  - Slight increase in size and minor EE loss
  - Still within acceptable limits
- Indicates **excellent physical and chemical stability**

3. CONCLUSION

This study successfully designed and optimized Oleanolic Acid-loaded Nanostructured Lipid Carriers (OA-NLCs) for brain-targeted therapy via the intranasal route. The key findings include:

- Enhanced solubility and bioavailability of Oleanolic Acid
- Nanoparticle size <150 nm and high entrapment efficiency (>85%)
- Controlled drug release up to 24 hours
- Efficient nose-to-brain delivery validated by ex vivo studies
- Superior cytotoxicity against glioblastoma cells, compared to free OA
- Safe and biocompatible formulation
- Stability confirmed under refrigerated conditions

The nose-to-brain delivery of OA using NLCs provides a non-invasive, site-specific, and effective platform for treating brain tumors like glioblastoma. Future studies may incorporate ligand-conjugated NLCs to further improve targeting and therapeutic index.

4. Discussion

The primary objective of this study was to formulate, optimize, and evaluate nanostructured lipid carriers (NLCs) for the targeted brain delivery of Oleanolic Acid (OA), a potent natural anticancer agent with limited clinical applicability due to its poor aqueous solubility, low bioavailability, and restricted BBB permeability.

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