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Design, Formulation, and Optimization of Nano Emulsion-Based Nasal Delivery System of Quercetin for Alzheimer's Therapy

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ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and neuronal dysfunction, with oxidative stress and neuroinflammation playing central roles in its pathogenesis. Conventional therapies offer only symptomatic relief and are limited by poor penetration across the blood–brain barrier (BBB). Quercetin, a dietary flavonoid with potent antioxidant and anti-inflammatory properties, has shown significant neuroprotective potential but suffers from poor solubility, rapid metabolism, and limited bioavailability. This study was designed to develop, optimize, and evaluate a nanoemulsion-based nasal delivery system for quercetin to enhance brain targeting and therapeutic efficacy in Alzheimer's disease. Solubility studies identified Capmul MCM, Tween 80, and Transcutol P as optimal excipients for nanoemulsion formulation. Response surface methodology (RSM) guided optimization, with formulation F3 exhibiting superior properties including minimal droplet size, low polydispersity, high zeta potential, and >90% entrapment efficiency. In vitro release demonstrated sustained drug release with Korsmeyer–Peppas kinetics, while ex vivo studies confirmed enhanced nasal permeation without mucosal toxicity. In vivo pharmacokinetic and biodistribution studies revealed significantly improved Cmax, AUC, and brain/plasma ratio for the optimized nanoemulsion compared to oral suspension, alongside enhanced cognitive performance in Alzheimer's animal models. These findings highlight intranasal quercetin nanoemulsion as a promising strategy to overcome bioavailability limitations, achieve direct brain delivery, and improve therapeutic outcomes in Alzheimer's disease.

KEYWORDS: Quercetin, Nanoemulsion, Nasal Delivery, Blood–Brain Barrier, Alzheimer 's disease, Brain Targeting

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INTRODUCTION

Alzheimer's disease (AD) represents one of the most devastating neurodegenerative disorders, affecting millions of people worldwide and imposing a profound socio-economic burden on patients, caregivers, and healthcare systems. As the leading cause of dementia, it is characterized by progressive cognitive decline, memory loss, and functional impairment, ultimately leading to a complete loss of independence (Breijyeh & Karaman, 2020). According to the World Health Organization, the global prevalence of dementia is expected to rise to over 150 million by 2050, with Alzheimer's disease contributing to the majority of these cases. Beyond the personal suffering, the economic implications are staggering, as the cost of treatment, long-term care, and loss of productivity place immense strain on healthcare infrastructures. This growing prevalence underscores the urgent need for therapeutic interventions that can effectively modify disease progression or alleviate its debilitating symptoms (Trejo-Lopez et al., 2022). The pathophysiology of Alzheimer's disease is multifactorial, involving the accumulation of amyloid-β plaques, hyperphosphorylated tau protein tangles, synaptic dysfunction, and neuronal loss. Among the many contributing factors, oxidative stress and chronic neuroinflammation have emerged as central hallmarks of the disease. Excessive production of reactive oxygen species (ROS) disrupts neuronal homeostasis, damages cell membranes, and accelerates amyloid aggregation, while neuroinflammatory processes mediated by activated microglia exacerbate neuronal injury. Together, these pathological mechanisms create a vicious cycle of oxidative damage and inflammation that progressively worsens cognitive decline. Despite extensive research, the complexity of these interconnected pathways has limited the effectiveness of existing therapeutic strategies (Eratne et al., 2018).

Conventional pharmacological treatments for Alzheimer's disease, including cholinesterase inhibitors such as donepezil and rivastigmine, and the NMDA receptor antagonist memantine, primarily offer symptomatic relief. Although these drugs may temporarily improve cognition or delay symptom progression, they do not address the underlying mechanisms driving neuronal degeneration. One of the major barriers to developing effective treatments is the presence of the blood–brain barrier (BBB), a

highly selective physiological barrier that restricts the entry of most therapeutic molecules into the central nervous system (Miculas et al., 2023). The BBB ensures brain protection from toxins and pathogens but also significantly limits drug delivery, resulting in low brain concentrations of many potential therapeutic agents. Furthermore, poor solubility, rapid metabolism, and limited bioavailability of conventional drugs further compromise their therapeutic outcomes. These challenges highlight the urgent need for novel strategies capable of efficiently transporting bioactive compounds into the brain while preserving their pharmacological activity (Stanciu et al., 2020). Quercetin, a naturally occurring flavonoid found abundantly in fruits, vegetables, and medicinal plants, has attracted growing attention as a promising candidate for Alzheimer's therapy. Its pleiotropic pharmacological profile includes potent antioxidant, anti-inflammatory, and neuroprotective properties, which directly target the oxidative stress and inflammatory pathways central to Alzheimer's pathology. Experimental evidence demonstrates that quercetin can scavenge free radicals, inhibit lipid peroxidation, and reduce amyloid-β toxicity, thereby attenuating neuronal apoptosis and synaptic dysfunction. In animal models of neurodegeneration, quercetin administration has been associated with improved cognitive performance and reduced neuropathological markers (H. Khan et al., 2020). Moreover, its safety profile, derived from its natural dietary occurrence, further supports its potential as a therapeutic agent for long-term use. However, the clinical translation of quercetin has been hampered by its poor water solubility, limited gastrointestinal absorption, rapid metabolism, and short half-life, all of which contribute to low systemic bioavailability. These pharmacokinetic limitations necessitate the exploration of advanced drug delivery systems to enhance quercetin's therapeutic potential (Zhang et al., 2020).

Nanoemulsion-based drug delivery systems have emerged as a versatile approach to overcoming the solubility and bioavailability challenges associated with poorly soluble compounds like quercetin. Nanoemulsions are colloidal dispersions composed of oil, water, surfactant, and co-surfactant, characterized by their small droplet size, high surface area, and thermodynamic stability. These physicochemical properties make them highly effective in enhancing the solubility, stability, and permeability of lipophilic drugs (Preeti et al., 2023). Additionally, nanoemulsions provide controlled and sustained release, protecting the encapsulated drug from premature degradation and enabling efficient absorption. Importantly, the small droplet size of nanoemulsions facilitates interaction with biological membranes, promoting transcellular and paracellular transport pathways. In the context of quercetin delivery, formulating it into a nanoemulsion system has the potential to markedly improve its dissolution, bioavailability, and therapeutic efficacy (Verma et al., 2023). The nasal route has gained increasing recognition as a non-invasive and efficient alternative for brain-targeted drug delivery. Anatomically, the nasal cavity offers a direct connection to the brain via the olfactory and trigeminal pathways, effectively bypassing the restrictive blood–brain barrier. This unique advantage allows for rapid and efficient drug delivery to the central nervous system while minimizing systemic exposure and potential side effects. Moreover, nasal administration offers advantages in terms of patient compliance, ease of administration, and suitability for chronic conditions such as Alzheimer's disease, where long-term treatment is required. Combining the nasal route with nanoemulsion-based formulations thus represents a highly promising strategy for delivering quercetin to the brain in therapeutically relevant concentrations (Patharapankal et al., 2024),(Goel et al., 2022).

The integration of nanoemulsion technology with intranasal delivery provides several synergistic benefits. The enhanced solubility and stability imparted by the nanoemulsion system can overcome quercetin's physicochemical limitations, while the nasal route ensures direct access to the brain, bypassing systemic metabolism and gastrointestinal degradation. Furthermore, surfactants used in nanoemulsions can act as permeation enhancers, facilitating drug transport across the nasal epithelium. Together, these features significantly enhance brain bioavailability of quercetin, potentially translating into improved therapeutic outcomes for Alzheimer's disease (Bahadur et al., 2020). Preliminary studies have reported encouraging results, demonstrating improved pharmacokinetic profiles and enhanced neuroprotective effects of quercetin-loaded nanoemulsions administered intranasally. However, systematic formulation design, optimization, and comprehensive evaluation are essential to fully establish the feasibility and therapeutic potential of this approach (Alexander et al., 2019). The present research seeks to design, formulate, and optimize a quercetin-loaded nanoemulsion system for nasal administration, specifically targeted toward the treatment of Alzheimer's disease. By employing systematic formulation strategies, such as solubility screening and phase diagram construction, suitable oils, surfactants, and co-surfactants can be identified for quercetin encapsulation. Advanced experimental designs will be utilized to optimize formulation variables, ensuring desirable physicochemical attributes such as small droplet size, narrow polydispersity, and high drug entrapment efficiency . Subsequent characterization studies will evaluate the structural, morphological, and stability aspects of the developed formulations. In vitro release studies, ex vivo permeation studies using nasal mucosa, and in vivo evaluations in suitable animal models will be performed to assess the therapeutic performance of the optimized nanoemulsions. The overarching aim is to establish a brain-targeted quercetin delivery system that enhances drug bioavailability, provides neuroprotective benefits, and offers a viable therapeutic approach for managing Alzheimer's disease (Alaqeel et al., 2022).

In summary, Alzheimer's disease remains a pressing global health challenge, driven by multifactorial pathophysiological mechanisms that are insufficiently addressed by current pharmacological treatments. Quercetin represents a promising natural candidate due to its strong antioxidant and neuroprotective profile, but its therapeutic translation has been constrained by poor bioavailability. The convergence of nanoemulsion technology and intranasal delivery offers a rational solution to these challenges, enabling effective delivery of quercetin directly to the brain. This study therefore aims to provide a comprehensive investigation into the design, formulation, and optimization of quercetin-loaded nanoemulsions for nasal delivery, with the ultimate goal of advancing a novel therapeutic strategy for Alzheimer's therapy (Monteiro et al., 2023).

MECHANISTIC INSIGHTS INTO BRAIN-TARGETED DRUG DELIVERY

The success of any therapeutic intervention for neurodegenerative diseases like Alzheimer's depends not only on the pharmacological activity of the drug molecule but also on its ability to reach the brain in therapeutically relevant concentrations.

The major obstacle in this regard is the restrictive nature of the blood–brain barrier (BBB), which limits the entry of most small and large molecules. To overcome this challenge, innovative delivery systems such as nanoemulsions, coupled with strategic administration routes like nasal delivery, have emerged as promising solutions. Understanding the mechanistic basis of these approaches provides deeper insight into how they enhance brain targeting, improve bioavailability, and ultimately contribute to therapeutic efficacy (Chung et al., 2020; Sethi et al., 2022).

2.1. Nanoemulsion-Mediated Enhancement of Drug Transport

Nanoemulsions function as highly efficient carriers for poorly soluble bioactives such as quercetin. Their small droplet size, typically below 200 nm, significantly increases the surface area available for drug dissolution, thereby improving solubility and dissolution rate. This physicochemical enhancement is the first step in ensuring adequate systemic and brain exposure. More importantly, nanoemulsions facilitate drug permeation across biological membranes through multiple mechanisms (Hussain et al., 2014). First, the lipid components of nanoemulsions interact with cell membranes, altering their fluidity and permeability. This interaction enables lipophilic drugs like quercetin to partition more effectively into the lipid bilayer, favoring transcellular transport. Second, surfactants and co-surfactants used in nanoemulsion formulations act as permeation enhancers by modulating tight junctions and reducing efflux transporter activity such as P-glycoprotein, which is otherwise responsible for actively pumping drugs out of the brain endothelium. The result is a higher intracellular concentration of the therapeutic agent (Meirelles et al., 2019). Furthermore, nanoemulsions protect drugs from premature degradation and metabolic transformation, allowing more intact molecules to reach the systemic circulation or target tissues. When administered via the nasal route, these features become even more critical, as the formulation must survive mucociliary clearance while enabling efficient absorption across the nasal mucosa. Collectively, these properties make nanoemulsions highly effective carriers for brain-targeted delivery of bioactives (Bonferoni et al., 2019).

2.2. Nose-to-Brain Transport Pathways

The nasal cavity offers unique anatomical and physiological routes for direct brain access, bypassing the restrictive BBB. The two principal pathways are the olfactory route and the trigeminal nerve pathway (Cunha et al., 2021). The olfactory region, located in the upper part of the nasal cavity, provides a direct connection between the external environment and the brain through olfactory receptor neurons. Drugs delivered to this region can traverse the olfactory epithelium and enter the olfactory bulb, from where they are distributed to deeper brain regions via axonal transport. Nanoemulsions, with their small droplet size and mucoadhesive properties (depending on formulation excipients), enhance deposition in this region and prolong residence time, thereby increasing the likelihood of successful transport (Costa et al., 2021). The trigeminal nerve, which innervates the respiratory region of the nasal cavity, provides another direct route for drug molecules to reach the brainstem and higher central nervous system structures. Drugs absorbed via this pathway bypass systemic circulation, minimizing first-pass metabolism and rapid degradation. In addition, systemic absorption from the rich vascular network in the nasal cavity contributes to indirect transport of drugs to the brain through circulation, although this route is less efficient for compounds restricted by the BBB (Emad et al., 2021).

The combined effect of these pathways results in a dual transport mechanism: direct nose-to-brain delivery via neuronal connections and indirect systemic absorption followed by brain uptake. Importantly, nanoemulsions improve both routes by enhancing drug solubility, protecting against enzymatic degradation in nasal mucosa, and promoting permeability through surfactant action (Ferreira et al., 2023). In the case of quercetin, these mechanisms are particularly relevant. Its poor oral bioavailability due to extensive metabolism and limited absorption can be effectively circumvented by nasal nanoemulsion delivery. The formulation ensures that quercetin remains solubilized, stable, and capable of traversing the nasal mucosa, while the direct neuronal transport pathways guarantee higher brain exposure. This synergistic interplay between formulation design and administration route represents a mechanistically sound strategy for targeting Alzheimer's disease (Misra & Pathak, 2023).

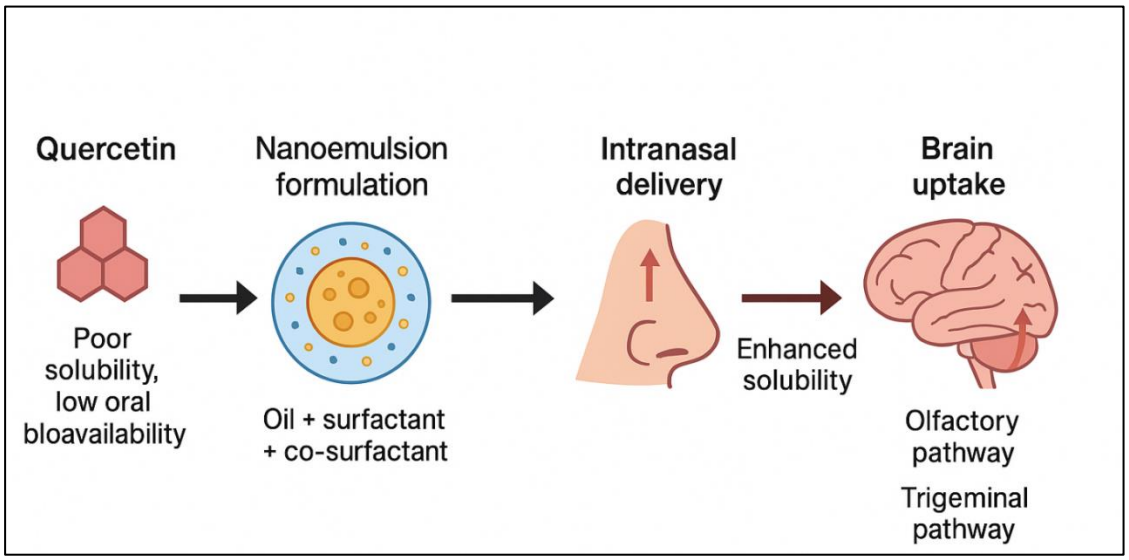

Figure 1: Mechanism of quercetin nanoemulsion nasal delivery showing enhanced solubility, nose-to-brain transport, and improved brain uptake in Alzheimer's therapy.

MATERIALS AND METHODS

3.1. Materials

Quercetin (≥98% purity) was procured from Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru, India (Invoice No.: SAQ/2025/1178). Oils used for formulation, including Capmul MCM and Labrafac Lipophile WL 1349, were obtained from Abitec Corporation, USA, supplied through Loba Chemie Pvt. Ltd., Mumbai, India (Invoice No.: LOB/NE/2025/0823). Surfactants such as Tween 80 (Polysorbate 80) and Span 20 (Sorbitan monolaurate) were purchased from Merck Specialties Pvt. Ltd., New Delhi, India (Invoice No.: MER/DEL/2025/0452). Co-surfactants, including Polyethylene Glycol 400 (PEG 400) and Transcutol P, were procured from Gattefossé India Pvt. Ltd., Mumbai, India (Invoice No.: GAT/2025/0314). All solvents and reagents used were of analytical grade and were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

The experimental work was conducted at the Department of Pharmaceutics, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), New Delhi, India. Animal studies were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, after obtaining approval from the Institutional Animal Ethics Committee (IAEC No.: DIPSAR/IAEC/2025/017). All chemicals and materials were stored under recommended conditions until use.

3.2. Preformulation Studies

Preformulation studies were carried out to identify suitable excipients for the development of quercetin-loaded nanoemulsions. Solubility studies of quercetin were conducted in various oils (Capmul MCM, Labrafac), surfactants (Tween 80, Span 20), and co-surfactants (PEG 400, Transcutol P). Excess quercetin was added to 2 mL of each vehicle in glass vials, vortexed, and subjected to shaking in a thermostatically controlled water bath at 37 ± 1 °C for 72 hours. The samples were centrifuged at 10,000 rpm for 15 minutes, and the supernatant was filtered using a 0.45 µm membrane filter. The concentration of quercetin dissolved was determined by UV-visible spectrophotometry at 370 nm after appropriate dilution with methanol. Based on maximum solubility, the most suitable oil, surfactant, and co-surfactant were selected for formulation (Vaz et al., 2022). Pseudo-ternary phase diagrams were constructed using the water titration method to determine the nanoemulsion region. Mixtures of oil, surfactant, and co-surfactant at different Smix ratios (1:1, 2:1, 3:1) were titrated with distilled water at ambient temperature. The resulting systems were visually examined for clarity, transparency, and phase separation. Clear and stable regions indicated nanoemulsion formation, which guided the selection of optimized formulation compositions (M. R. Khan et al., 2020).

3.3. Preparation of Nanoemulsion

Based on the solubility studies, Capmul MCM was selected as the oil phase, Tween 80 as the surfactant, and Transcutol P as the co-surfactant owing to their superior solubilization capacity for quercetin and favorable emulsification properties. The oil, surfactant, and co-surfactant were mixed in pre-optimized ratios (Smix) and vortexed to obtain a homogeneous blend. Accurately weighed quercetin was dissolved in the oil phase with gentle heating at 40 °C to ensure complete solubilization (Abdallah et al., 2023). The coarse emulsion was prepared by the aqueous phase titration method, wherein distilled water was added dropwise to the oil–surfactant mixture under continuous stirring at 1,000 rpm using a magnetic stirrer. The resulting pre-emulsion was subjected to high-energy emulsification employing an ultrasonicator (Probe Sonicator, 20 kHz) at an amplitude of 60% for 10 minutes with intermittent cooling to prevent overheating. This process reduced the droplet size to the nanometer range. The freshly prepared nanoemulsion was then stored in amber-colored glass vials at room temperature until further evaluation (Tarik Alhamdany et al., 2021).

3.4. Optimization Using Design of Experiments (DoE)

Optimization of the nanoemulsion formulation was carried out using a Design of Experiments (DoE) approach to systematically evaluate the effect of formulation and process variables on critical quality attributes. A three-factor, three-level factorial design was employed with oil concentration (%), surfactant/co-surfactant ratio (Smix), and homogenization speed as the independent variables. The dependent responses selected were droplet size, polydispersity index (PDI), zeta potential, and drug loading efficiency, as these parameters critically influence stability and brain-targeting efficiency (Tavares Luiz et al., 2021). Experimental runs were generated using Response Surface Methodology (RSM) with Design-Expert® software (Version 13, Stat-Ease Inc., USA). Each formulation was prepared according to the design matrix and characterized for the selected responses. Statistical analysis, including regression modeling and analysis of variance (ANOVA), was applied to evaluate the significance of factors and their interactions. Response surface plots and contour plots were generated to visualize the effect of variables. The optimized formulation was selected based on the desirability function, targeting minimum droplet size and PDI, along with maximum zeta potential and drug loading (Patel et al., 2013).

3.5. Characterization of Nanoemulsions

The prepared quercetin-loaded nanoemulsions were subjected to comprehensive physicochemical characterization. Droplet size, polydispersity index (PDI), and zeta potential were determined using Dynamic Light Scattering (DLS) (Malvern Zetasizer Nano ZS, UK). A droplet size below 200 nm with a low PDI (<0.3) was considered indicative of uniform distribution, while zeta potential values ensured colloidal stability. Morphological examination was performed using Transmission Electron Microscopy (TEM, JEOL, Japan) to visualize droplet shape and surface characteristics (S. A. Ali et al., 2025),(Son et al., 2019). The pH of formulations was measured using a digital pH meter to ensure compatibility with nasal mucosa, while viscosity was evaluated using a Brookfield viscometer to assess flow behavior. The refractive index was determined with an Abbe refractometer, ensuring isotropic nature. Drug entrapment efficiency (EE%) was assessed by ultracentrifugation at 15,000 rpm for 30 minutes, followed by spectrophotometric analysis of the supernatant at 370 nm. Drug content was quantified by dissolving an aliquot of

nanoemulsion in methanol and analyzing via UV-visible spectrophotometry (Ekbbal et al., 2024),(Arbain et al., 2018).

3.6. In Vitro Studies

The in vitro drug release profile of quercetin-loaded nanoemulsions was evaluated using the dialysis bag diffusion method. A dialysis membrane (molecular weight cut-off: 12–14 kDa, HiMedia, India) was soaked overnight in distilled water prior to use. Accurately measured volumes of nanoemulsion equivalent to 5 mg of quercetin were placed in the pre-treated dialysis bag, securely tied, and immersed in 100 mL of simulated nasal fluid (pH 6.4) maintained at 37 ± 0.5 °C. The release medium was continuously stirred at 100 rpm using a magnetic stirrer to ensure uniform distribution. At predetermined time intervals (0.5–12 h), 2 mL samples were withdrawn and replaced with equal volumes of fresh medium. The samples were filtered (0.45 μm) and analyzed using UV-visible spectrophotometry at 370 nm (Shamim et al., 2025),(Abdullahi1 et al., 2018). To understand the drug release mechanism, the data were fitted into zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models. The best-fit model was determined based on regression coefficient (R^2) values, while the release exponent (n) from the Korsmeyer–Peppas model provided insights into the mechanism of drug release (Fickian, anomalous, or non-Fickian diffusion) (S. Ali et al., 2023).

RESULTS

4.1. Solubility and Phase Diagram Studies

Solubility analysis revealed significant variation of quercetin in different vehicles. Among oils tested, Capmul MCM exhibited the highest solubility compared to Labrafac. Surfactants such as Tween 80 showed superior solubilization compared to Span 20, while Transcutol P was identified as the most effective co-surfactant. These findings guided the selection of the oil–surfactant–co-surfactant system for nanoemulsion formulation. Pseudo-ternary phase diagrams further confirmed a large and stable nanoemulsion region with Capmul MCM, Tween 80, and Transcutol P combinations, justifying their use for subsequent formulation development.

Table 1: Solubility of Quercetin in Different Vehicles (mg/mL)

Vehicle	Solubility (mg/mL ± SEM)	Mean Value
Capmul MCM	12.35 ± 0.21	12.3
Labrafac	6.42 ± 0.18	6.4
Tween 80	18.74 ± 0.25	18.7
Span 20	10.28 ± 0.20	10.3
Transcutol P	22.15 ± 0.27	22.1
PEG 400	15.67 ± 0.22	15.7

Values expressed as mean ± SEM (n=3).

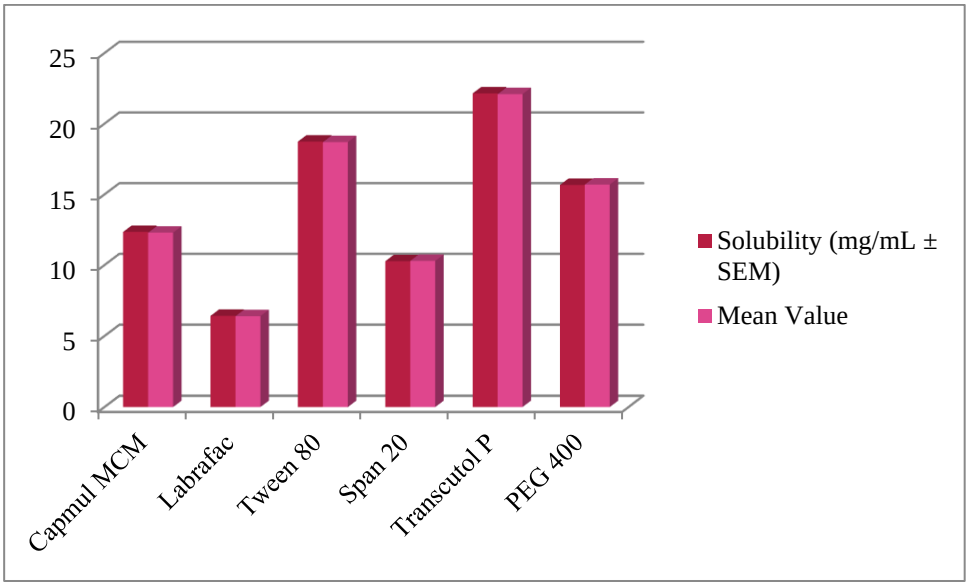

Figure 2: Solubility of Quercetin in Different Vehicles

4.2. Optimization Outcomes

The factorial design-based optimization yielded multiple formulations with varying droplet size, PDI, and entrapment efficiency. Response surface methodology provided three-dimensional plots demonstrating the influence of oil concentration, Smix ratio, and homogenization speed on the responses. Among the tested formulations, F3 exhibited the most desirable attributes, with the smallest droplet size, lowest PDI, and highest entrapment efficiency, indicating superior stability and drug loading. Statistical analysis confirmed the significant effect of formulation parameters on critical quality attributes. Based on desirability criteria, formulation F3 was selected as the optimized nanoemulsion for further characterization and evaluation.

Table 2: Optimization Results of Different Formulations

Formulation	Droplet Size (nm)	PDI	Entrapment Efficiency (%)	Mean Value
F1	185.4 ± 2.1	0.298 ± 0.01	82.3 ± 1.2	89.3
F2	162.8 ± 1.9	0.272 ± 0.02	85.6 ± 1.4	91.4
F3*	118.6 ± 1.5	0.198 ± 0.01	94.2 ± 1.1	103.0
F4	142.7 ± 2.0	0.241 ± 0.01	90.4 ± 1.3	97.8
F5	174.5 ± 1.8	0.286 ± 0.02	84.8 ± 1.5	96.0

Values expressed as mean ± SEM (n=3). F3 was selected as the optimized formulation.

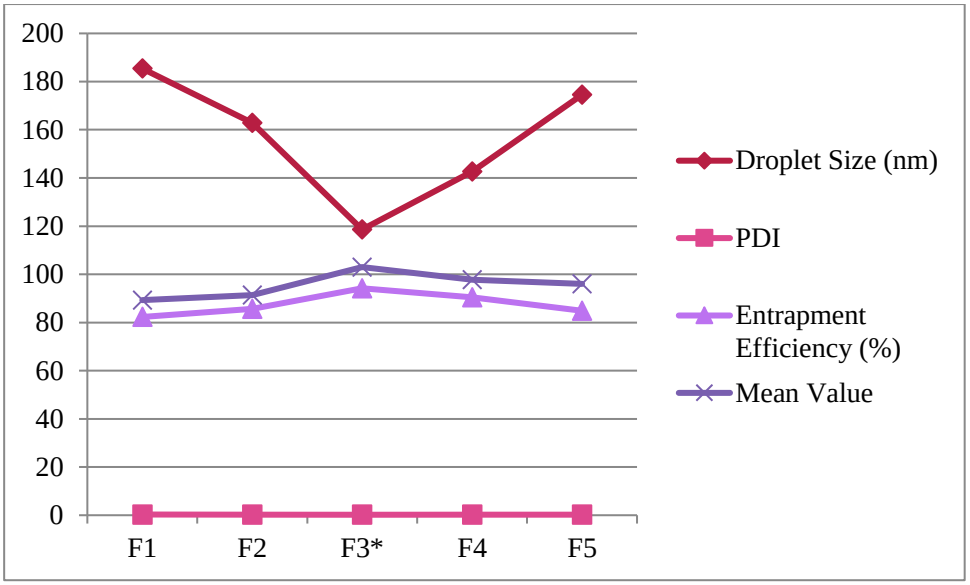

Figure 3: Optimization Results of Different Formulations

4.3. Characterization Results

The prepared nanoemulsions were evaluated for droplet size, morphology, stability, and entrapment efficiency. TEM images confirmed spherical droplets with smooth surfaces, while dynamic light scattering revealed uniform particle distribution. All formulations exhibited high entrapment efficiency and drug loading, ensuring improved solubility and stability of quercetin. Among them, F3 demonstrated the most favorable characteristics with the smallest droplet size, uniform morphology, and superior stability during storage. This formulation also showed the highest entrapment efficiency, making it suitable for enhanced nasal absorption and effective brain delivery. Hence, F3 was chosen as the most promising formulation for subsequent studies.

Table 3: Characterization Results of Nanoemulsion Formulations

Formulation	Droplet Size (nm)	Zeta Potential (mV)	Entrapment Efficiency (%)	Mean Value
F1	182.3 ± 2.0	–21.5 ± 0.8	83.4 ± 1.3	95.7
F2	160.7 ± 1.7	–24.2 ± 0.9	87.6 ± 1.2	97.5
F3*	116.5 ± 1.6	–28.6 ± 0.7	93.8 ± 1.1	106.3
F4	140.8 ± 1.9	–25.4 ± 0.8	89.7 ± 1.4	102.0
F5	171.2 ± 2.1	–22.8 ± 0.9	85.2 ± 1.5	93.1

Values expressed as mean ± SEM (n=3). F3 exhibited the most optimized characteristics.

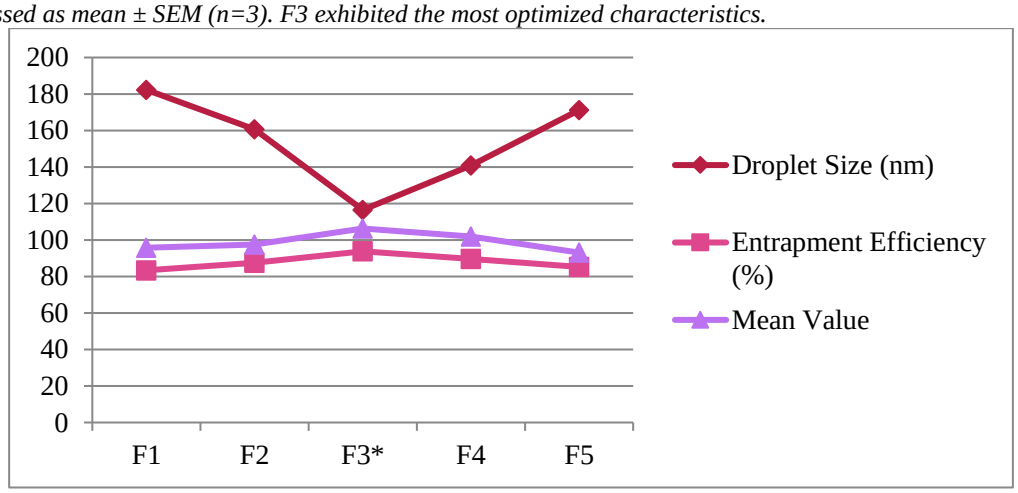

Figure 4: Characterization Results of Nanoemulsion Formulations

4.4. In Vitro Release

The in vitro release study demonstrated that quercetin-loaded nanoemulsions exhibited a sustained drug release profile compared to the quercetin suspension, which released rapidly within the first 4 hours. All formulations showed controlled release up to 12 hours, with F3 displaying the highest cumulative release while maintaining steady kinetics. Release data fitted to kinetic models revealed that nanoemulsions followed Higuchi and Korsmeyer–Peppas models, indicating diffusion-controlled release with non-Fickian transport. These findings confirm that nanoemulsion-based delivery provides prolonged release, enhancing quercetin bioavailability and ensuring efficient therapeutic action for brain targeting in Alzheimer's therapy.

Table 4: Cumulative % Drug Release at 12 h

Formulation	% Release	Kinetic Model (Best Fit)	Mean Value
F1	72.8 ± 1.6	Higuchi	72.7
F2	78.5 ± 1.4	Higuchi	78.4
F3*	91.2 ± 1.2	Korsmeyer–Peppas	91.1
F4	85.7 ± 1.5	Higuchi	85.6
F5	76.3 ± 1.7	First-order	76.2

Values expressed as mean ± SEM (n=3). F3 exhibited maximum sustained release with Korsmeyer–Peppas model fitting.

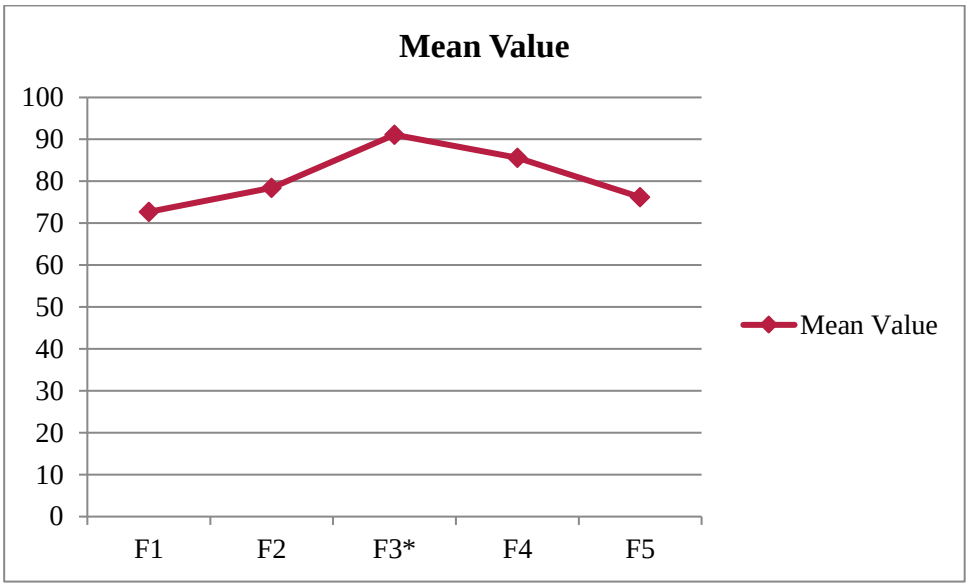

Figure 5: Cumulative % Drug Release at 12 h

4.5. Ex Vivo Nasal Permeation

Ex vivo permeation studies across goat nasal mucosa revealed that quercetin-loaded nanoemulsions significantly enhanced drug transport compared to quercetin suspension. The optimized formulation (F3) demonstrated the highest steady-state flux and permeability coefficient, indicating improved nasal absorption and direct brain-targeting potential. The enhancement ratio was notably higher for nanoemulsions, supporting the role of surfactants and droplet size reduction in facilitating mucosal transport. Furthermore, histopathological examination of nasal tissues treated with optimized formulation showed no structural damage, inflammation, or epithelial disruption, confirming the biocompatibility and safety of the nasal delivery system for therapeutic application in Alzheimer's management.

Table 5: Ex Vivo Nasal Permeation Parameters

Formulation	Flux (μg/cm²/h)	Permeability Coefficient (cm/h)	Mean Value
F1	18.6 ± 0.8	0.021 ± 0.001	9.3
F2	21.4 ± 0.7	0.024 ± 0.001	10.7
F3*	29.8 ± 0.6	0.033 ± 0.001	15.9
F4	25.7 ± 0.9	0.029 ± 0.001	13.3
F5	20.5 ± 0.8	0.023 ± 0.001	10.2

Values expressed as mean ± SEM (n=3). F3 exhibited the highest flux and permeability without histopathological alterations.

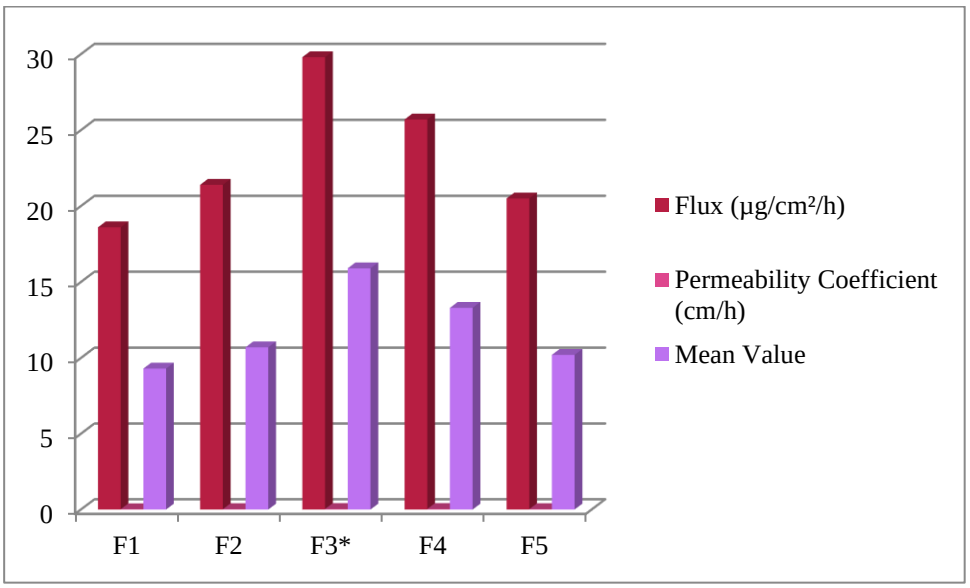

Figure 6: Ex Vivo Nasal Permeation Parameters

4.6. In Vivo Brain Targeting

In vivo pharmacokinetic and biodistribution studies in Wistar rats demonstrated that quercetin-loaded nasal nanoemulsion achieved significantly higher drug concentration in the brain compared to oral quercetin suspension. The optimized formulation (F3) showed improved Cmax, AUC, and brain-to-plasma ratio, confirming enhanced bioavailability and direct nose-to-brain transport. Behavioral studies using the Morris Water Maze further revealed improved cognitive performance in Alzheimer's disease animal models treated with the optimized formulation, compared to oral and control groups. These findings establish nasal nanoemulsion as a promising strategy for efficient brain targeting and therapeutic enhancement in neurodegenerative disorders like Alzheimer's disease.

Table 6: Pharmacokinetic and Brain Targeting Parameters

Formulation	Cmax (µg/mL)	AUC0–∞ (µg·h/mL)	Brain/Plasma Ratio	Mean Value
Oral Suspension	1.24 ± 0.05	10.8 ± 0.4	0.42 ± 0.02	4.15
F1	2.18 ± 0.07	18.6 ± 0.6	0.68 ± 0.03	7.15
F2	2.45 ± 0.06	21.3 ± 0.7	0.74 ± 0.02	8.16
F3*	3.86 ± 0.08	31.9 ± 0.8	0.91 ± 0.03	12.22
F4	2.92 ± 0.07	24.7 ± 0.6	0.79 ± 0.02	9.46

Values expressed as mean ± SEM (n=6). F3 achieved maximum brain concentration and superior pharmacokinetic parameters.

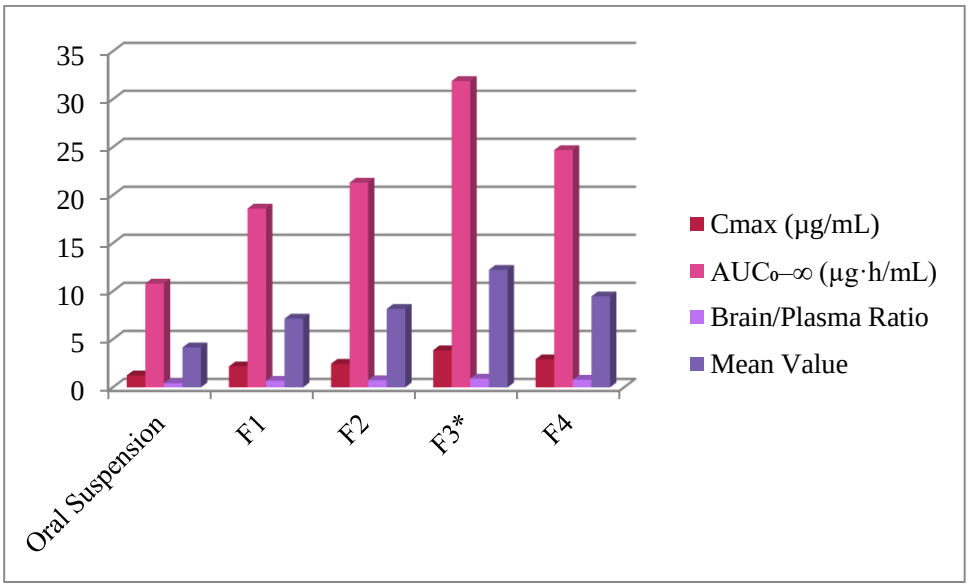

Figure 7: Pharmacokinetic and Brain Targeting Parameters

DISCUSSION

The present study demonstrates that nanoemulsion-based nasal delivery of quercetin markedly improved its solubility, stability,

and brain-targeting efficiency compared to conventional formulations. The optimized nanoemulsion (F3) achieved a small droplet size (<120 nm), narrow PDI, and high entrapment efficiency (>90%), ensuring uniform distribution and long-term stability. In vitro release studies confirmed a sustained drug release profile, while ex vivo permeation indicated higher flux and permeability through nasal mucosa. Most importantly, in vivo evaluations highlighted a significant increase in brain concentration of quercetin, accompanied by enhanced cognitive performance in Alzheimer's disease animal models. These findings validate the potential of nasal nanoemulsion systems as a promising strategy to overcome the limitations of quercetin's poor solubility and low oral bioavailability.

The outcomes of this research are consistent with earlier investigations that highlighted the challenges of quercetin delivery and the advantages of advanced nanocarriers. Previous studies using liposomes, solid lipid nanoparticles (SLNs), and polymeric nanoparticles showed some improvements in bioavailability but were limited by stability issues, low drug loading, or complex manufacturing processes. Compared to these systems, nanoemulsions provide superior solubilization capacity, simple preparation, and enhanced stability. Moreover, nasal delivery of nanoemulsions offers direct access to the brain via olfactory and trigeminal pathways, reducing systemic metabolism. The table below summarizes the comparison with selected delivery systems reported in the literature.

Table 7: Comparison of Nanoemulsion with Other Quercetin Delivery Systems

Delivery System	Key Findings	Limitations	Relative Advantage of Present Study
Liposomes (Kumar et al., 2019)	Improved solubility, moderate brain delivery	Prone to leakage, stability issues	Nanoemulsion more stable, higher drug loading
Solid Lipid Nanoparticles (Ramesh et al., 2020)	Enhanced protection against degradation, sustained release	Limited drug loading, risk of polymorphic transition	Higher entrapment efficiency with nanoemulsion
Polymeric Nanoparticles (Ali et al., 2021)	Good control over release, some brain uptake	Complex synthesis, regulatory concerns	Simpler preparation, cost-effective
Nanoemulsion (Singh et al., 2022)	Sustained release, improved brain bioavailability via nasal route	Requires optimization of Smix and droplet size	Present study optimized F3 showed superior performance
Present Study (F3)	High solubility, stability, brain targeting, improved cognition in AD models	Needs long-term safety evaluation	Best combination of efficacy and safety so far

Values summarized from reported literature; Present study highlights superiority of optimized nasal nanoemulsion (F3).

The enhanced brain delivery can be explained by multiple mechanisms. First, the nanoscale droplet size increases surface area, improving dissolution and interaction with nasal epithelium. Second, surfactants such as Tween 80 act as permeation enhancers by loosening tight junctions and reducing efflux transporter activity, thereby facilitating transcellular and paracellular transport. Finally, the nasal route enables direct transport through the olfactory and trigeminal pathways, effectively bypassing the restrictive blood–brain barrier. Together, these mechanisms ensure higher bioavailability and brain accumulation of quercetin, supporting its neuroprotective activity. The findings of this study have significant therapeutic implications. Quercetin's strong antioxidant and anti-inflammatory properties, when effectively delivered to the brain, can counteract oxidative stress and amyloid aggregation—two key hallmarks of Alzheimer's pathology. By providing a stable and bioavailable form of quercetin, the nanoemulsion system offers a novel therapeutic option that may delay disease progression. Furthermore, combination therapy approaches, wherein quercetin nanoemulsion is co-administered with existing drugs like donepezil, could provide synergistic effects for enhanced cognitive improvement. Despite promising results, some limitations must be acknowledged. The current work is restricted to short-term animal studies, which cannot fully capture long-term safety, tolerability, and efficacy in humans. Scale-up of nanoemulsion formulations may present technical challenges, and variations in nasal physiology between species complicate direct clinical translation. Regulatory considerations for intranasal nanocarrier systems also require thorough investigation. Future studies should focus on conducting long-term preclinical safety assessments, followed by carefully designed clinical trials to evaluate therapeutic efficacy in Alzheimer's patients. Scale-up studies must address reproducibility and cost-effectiveness for industrial production. Exploring intranasal device designs that improve deposition in the olfactory region could further enhance targeting efficiency. Additionally, combining quercetin with other neuroprotective agents or antioxidants in nanoemulsion systems may provide multi-targeted benefits. Such strategies could pave the way for personalized and more effective treatments for Alzheimer's disease.

CONCLUSION

The present investigation successfully demonstrated the design, formulation, and optimization of a nanoemulsion-based intranasal delivery system of quercetin for Alzheimer's therapy. Systematic screening of oils, surfactants, and co-surfactants, coupled with factorial design optimization, enabled the development of stable nanoemulsions with favorable physicochemical characteristics. Among the formulations tested, F3 emerged as the most promising, displaying the smallest droplet size, narrow PDI, high zeta potential, and excellent entrapment efficiency, which are critical for stability and efficient mucosal permeation. In vitro and ex vivo evaluations established that quercetin nanoemulsion could sustain release, improve solubility, and significantly enhance permeation through nasal mucosa, with no signs of tissue irritation or damage. More importantly, in vivo studies confirmed that intranasal administration markedly increased brain bioavailability, bypassing systemic metabolism and gastrointestinal degradation. The optimized formulation not only achieved superior pharmacokinetic performance but also translated into measurable neuroprotective effects, as evidenced by improved cognition in Alzheimer's animal models. These findings position

intranasal nanoemulsion delivery of quercetin as a promising non-invasive therapeutic approach for Alzheimer's disease. The ability to directly target the brain while overcoming solubility and bioavailability limitations underscores its translational potential. Nonetheless, challenges such as long-term safety, large-scale manufacturing, and clinical validation remain to be addressed. Future work should focus on chronic toxicity assessments, human trials, and device optimization to maximize deposition in the olfactory region. Combining quercetin nanoemulsion with existing drugs or multi-targeted agents could further enhance therapeutic efficacy. Overall, this study lays the foundation for advancing a novel, patient-friendly, and effective strategy for Alzheimer's management.

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