Development, Physicochemical Characterization, and Neuroprotective Evaluation of Transferrin-Conjugated Nanostructured Lipid Carriers Loaded with Synthetic Pyrimidine Derivatives for Enhanced Brain Delivery in Alzheimer's Disease

Sushama Vaishnav[1], Rakh Limbraj B.[2], Dhirajkumar Nikam[3], Riya Jaiswal[4], Abhishek Ghosh[5], Preeti Juyal[6], Suneel Kumar[7], Geeta Yadav[8], Chaitali Chandrakant Teli[9*]

[1]Associate Professor, Department of Pharmaceutical Quality Assurance, Yash Institute of Pharmacy, Chhatrapati Sambhajinagar, Maharashtra, South City, Waluj Road, Opp. A. S. Club, Chhatrapati Sambhajinagar 431136, Maharashtra, India

[2]Assistant Professor, Department of Pharmacognosy, Dayanand Education Society's, Dayanand College of Pharmacy, Latur, Maharashtra, India

[3]Assistant Professor, Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Navi Mumbai, Thane, Maharashtra, India

[4]Assistant Professor, School of Pharmacy and Research, Devbhoomi Uttarakhand University, Chakrata Road, Navgaon, Manduwala, Dehradun, Uttarakhand, India

[5]Assistant Professor, School of Pharmacy, ARKA JAIN University, Mohanpur, Gamharia, Seraikela Kharsawan, Jamshedpur, Jharkhand - 832108, India

[6]Assistant Professor, Department of Microbiology, Shri Guru Ram Rai University, Dehradun, India

[7]Assistant Professor, Department of Pharmacology, Mangalayatan Institute of Pharmaceutical Education and Research, Mangalayatan University, Aligarh Mathura Aligarh Road, Beswan, Aligarh, India

[8]Professor, Department of Pharmacy, School of Pharmaceutical Sciences, CGC University, Mohali, Punjab, 140307, India

[9]Assistant Professor, Department of Pharmaceutics, K.B.H.S.S. Trust's Institute of Pharmacy, Malegaon, Nashik, Opp. Jajuwadi, Bhaygaon Road, Malegaon Camp, Dist-Nashik, Maharashtra, India

***Corresponding author: Chaitali Chandrakant Teli, Assistant Professor, Department of Pharmaceutics, K.B.H.S.S. Trust's Institute of Pharmacy, Malegaon, Nashik, Opp. Jajuwadi, Bhaygaon Road, Malegaon Camp, Dist-Nashik, Maharashtra, India**

Email: chaitalicteli2611@gmail.com

Received: 31st May, 2026; **Revised:** 8th June, 2026; **Accepted:** 10th June, 2026; **Available Online:** 12th June, 2026

ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment, neuronal degeneration, amyloid-beta accumulation, oxidative stress, and neuroinflammation, creating significant challenges for effective therapeutic intervention. Conventional treatment strategies are often limited by poor blood-brain barrier (BBB) penetration, inadequate target specificity, low bioavailability, and insufficient therapeutic concentrations within brain tissues. Therefore, the present study aimed to develop transferrin-conjugated nanostructured lipid carriers (Tf-NLCs) loaded with synthetic pyrimidine derivatives for enhanced brain delivery and improved neuroprotective efficacy in Alzheimer's disease. Drug-loaded nanostructured lipid carriers were successfully prepared using hot homogenization followed by ultrasonication and subsequently surface-functionalized with transferrin to facilitate receptor-mediated BBB transport. The developed formulations were subjected to extensive physicochemical characterization including particle size analysis, polydispersity index determination, zeta potential measurement, morphological evaluation, entrapment efficiency assessment, and in vitro release studies. The optimized formulation exhibited nanosized particles with narrow size distribution, favorable surface charge characteristics, high drug encapsulation efficiency, and sustained release behavior. BBB permeability and cellular uptake studies demonstrated significantly enhanced transport efficiency and improved neuronal internalization for transferrin-conjugated formulations compared with non-targeted systems. Neuroprotective evaluation further revealed improved behavioral performance, reduction in oxidative stress biomarkers, attenuation of neuroinflammatory responses, preservation of neuronal architecture, and decreased pathological alterations in treated groups. Histopathological findings confirmed reduced neuronal degeneration and improved tissue morphology following administration of optimized formulations. Overall, the results suggest that transferrin-conjugated nanostructured lipid carriers loaded with synthetic pyrimidine derivatives represent a promising targeted delivery platform capable of enhancing brain accumulation and improving therapeutic outcomes in Alzheimer's disease. This approach may contribute toward development of advanced nanotechnology-based interventions for effective management of neurodegenerative disorders.

Keywords: Alzheimer's disease, Nanostructured lipid carriers, Transferrin conjugation, Blood-brain barrier, Synthetic pyrimidine derivatives, Brain targeting, Neuroprotection, Nanomedicine, Drug delivery, Neurodegenerative disorders.

How to cite this article: Vaishnav S, Limbraj B R, Nikam D, Jaiswal R, Ghosh A, Juyal P, Kumar S, Yadav G, Teli CC. Development, Physicochemical Characterization, and Neuroprotective Evaluation of Transferrin-Conjugated Nanostructured Lipid Carriers Loaded with Synthetic Pyrimidine Derivatives for Enhanced Brain Delivery in Alzheimer's Disease. Int J Drug Deliv Technol. 2026;16(58s): 1429-1437. DOI: 10.25258/ijddt.16.58s.152
Source of support: Nil
Conflict of interest: None

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by gradual cognitive decline, impairment of memory, behavioral abnormalities, and deterioration of functional abilities, ultimately leading to complete dependence and mortality. AD represents the most common cause of dementia worldwide and has emerged as one of the greatest healthcare challenges due to the rapidly aging population. The prevalence of AD continues to rise globally, imposing substantial socioeconomic burdens on healthcare systems, caregivers, and society. Current estimates suggest that millions of individuals suffer from dementia worldwide, with Alzheimer's disease accounting for approximately 60–70% of all cases. The pathological progression of AD is associated with extracellular accumulation of amyloid-beta plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau proteins, oxidative stress, mitochondrial dysfunction, neuroinflammation, synaptic degeneration, and neuronal loss, ultimately resulting in progressive cognitive impairment (Breijyeh & Karaman, 2020).

The multifactorial nature of AD makes therapeutic intervention highly challenging because multiple molecular pathways contribute simultaneously to disease progression. Conventional pharmacological treatments including acetylcholinesterase inhibitors and NMDA receptor antagonists primarily provide symptomatic relief rather than modifying disease progression. Furthermore, currently available therapies suffer from poor brain penetration, limited bioavailability, short circulation time, systemic adverse effects, and inability to achieve adequate drug concentrations at target neuronal tissues. These limitations emphasize the urgent need for advanced therapeutic strategies capable of improving targeted drug delivery and enhancing therapeutic efficacy in neurodegenerative disorders (Mehta et al., 2019).

One of the greatest obstacles in treating neurological disorders is the presence of the blood-brain barrier (BBB), a highly selective physiological barrier that regulates molecular transport between systemic circulation and brain tissue. The BBB is composed of tightly connected endothelial cells, astrocytes, pericytes, and basement membrane structures that collectively restrict the entry of most therapeutic agents into the central nervous system. Approximately 98% of small molecules and nearly all macromolecules exhibit limited BBB permeability, resulting in insufficient drug accumulation within brain tissue. Additionally, conventional dosage forms often experience rapid clearance, enzymatic degradation, and extensive first-pass metabolism, further reducing therapeutic effectiveness. Consequently, targeted brain drug delivery systems have become increasingly important for overcoming BBB-associated limitations and improving treatment outcomes in AD (Pardridge, 2022).

Nanotechnology-based drug delivery systems have emerged as promising alternatives for enhancing CNS drug delivery due to their ability to improve drug stability, increase bioavailability, prolong circulation time, and facilitate targeted delivery. Among various nanosystems, nanostructured lipid carriers (NLCs) have gained considerable attention because they combine solid and liquid lipids to create imperfect lipid matrices capable of accommodating larger quantities of drug molecules. NLCs possess several advantages including biocompatibility, biodegradability, controlled drug release, enhanced stability, reduced toxicity, and improved encapsulation efficiency. Their nanoscale size enables increased interaction with biological membranes, thereby facilitating improved transport across physiological barriers. Furthermore, surface engineering strategies can significantly enhance the targeting capabilities of NLCs, making them particularly attractive for CNS drug delivery applications (Müller et al., 2016).

Surface modification of nanocarriers with targeting ligands represents an advanced strategy for enhancing BBB transport and improving brain accumulation of therapeutic agents. Among various targeting approaches, transferrin-mediated targeting has attracted considerable interest because transferrin receptors are abundantly expressed on BBB endothelial cells and neuronal tissues. Transferrin receptors participate in receptor-mediated endocytosis, allowing transport of iron-bound transferrin molecules from systemic circulation into brain tissue. By conjugating transferrin molecules onto nanoparticle surfaces, nanocarriers can exploit endogenous receptor-mediated transport mechanisms to cross the BBB more efficiently. This strategy has demonstrated

improved cellular uptake, enhanced brain targeting efficiency, prolonged circulation time, and increased therapeutic accumulation at pathological sites, thereby offering substantial advantages over passive diffusion-based delivery approaches (Ulbrich et al., 2011).

Synthetic pyrimidine derivatives have attracted growing attention as potential therapeutic candidates for neurodegenerative disorders because of their diverse pharmacological activities including antioxidant, anti-inflammatory, acetylcholinesterase inhibitory, anti-amyloidogenic, and neuroprotective properties. Pyrimidine-containing compounds exhibit favorable molecular characteristics that enable interaction with multiple pathological targets involved in AD progression. Recent investigations have highlighted the therapeutic potential of synthetic heterocyclic compounds, particularly pyrimidine and chalcone-based derivatives, in modulating pathways associated with amyloid aggregation, oxidative damage, and neuroinflammation. Structure-activity relationship studies have demonstrated that chemical modifications of these scaffolds significantly influence biological activity, supporting continued exploration of synthetic derivatives for AD treatment (Prabha & Rajput, 2025).

Despite promising biological activities, synthetic pyrimidine derivatives frequently suffer from poor aqueous solubility, inadequate pharmacokinetic profiles, limited BBB penetration, and insufficient target specificity. Encapsulation of these compounds within transferrin-conjugated NLCs may overcome such limitations by improving solubility, protecting drugs from premature degradation, enhancing controlled release behavior, and facilitating receptor-mediated transport across the BBB. Therefore, the present study aims to develop transferrin-conjugated nanostructured lipid carriers loaded with synthetic pyrimidine derivatives, followed by comprehensive physicochemical characterization and evaluation of neuroprotective efficacy for enhanced brain delivery in Alzheimer's disease. The study intends to investigate whether combining ligand-mediated targeting with nanostructured lipid-based delivery can provide improved therapeutic outcomes and represent a promising strategy for future AD management.

2. Materials and Methods

2.1 Materials

Synthetic pyrimidine derivatives utilized in the present investigation were synthesized and purified prior to formulation studies according to established synthetic procedures. The synthesized compounds were characterized using appropriate analytical methods before formulation development. Solid lipids including glyceryl monostearate, stearic acid, and cetyl palmitate, along with liquid lipids such as oleic acid or medium-chain triglycerides, were selected for the preparation of nanostructured lipid carriers based on their compatibility with the drug and ability to produce stable lipid matrices. Surfactants including polysorbate 80, poloxamer 188, and soy lecithin were employed for stabilization of nanoparticle dispersions. Human transferrin was used as the targeting ligand for surface conjugation studies.

All solvents and analytical reagents employed throughout the investigation were of analytical or HPLC grade. Cell culture reagents including fetal bovine serum, culture media, antibiotics, and assay reagents were procured from certified suppliers. In vitro studies utilized neuronal cell lines including SH-SY5Y neuroblastoma cells and brain endothelial cell models for evaluating BBB permeability. Experimental animals used for neuroprotective evaluation consisted of healthy adult rodents maintained under controlled environmental conditions with free access to food and water. Animal experiments were conducted according to institutional ethical guidelines and approved experimental protocols.

2.2 Preparation of Drug-Loaded Nanostructured Lipid Carriers

Drug-loaded nanostructured lipid carriers were prepared using a combination of hot homogenization and ultrasonication techniques. Briefly, selected solid lipids were melted at temperatures approximately 5–10°C above their melting point, followed by incorporation of liquid lipids to generate a homogenous lipid phase. Synthetic pyrimidine derivatives were dissolved or dispersed within the molten lipid mixture under continuous stirring.

An aqueous phase containing surfactants was heated to the same temperature as the lipid phase and gradually added under high-speed homogenization to form a coarse emulsion. The pre-emulsion was subjected to probe ultrasonication for nanoparticle size reduction. The resulting nanoemulsion was cooled to room temperature under continuous stirring to allow recrystallization of lipids and formation of nanostructured lipid carriers.

Optimization of formulation variables was performed using experimental design approaches. Independent variables including lipid concentration, surfactant concentration, liquid lipid proportion,

homogenization speed, and sonication time were systematically varied to identify optimized conditions capable of producing nanoparticles with desirable particle size, stability, and drug entrapment efficiency.

2.3 Surface Conjugation with Transferrin

Surface conjugation of nanostructured lipid carriers with transferrin was performed using carbodiimide-mediated coupling chemistry. Functional groups present on the nanoparticle surface were activated using coupling agents including N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide. Activated nanoparticles were incubated with transferrin solution under controlled reaction conditions to facilitate covalent attachment.

Following conjugation, unbound transferrin molecules were removed by repeated centrifugation and washing procedures. Surface-modified nanoparticles were collected and stored under refrigerated conditions until further analysis.

Successful conjugation was confirmed using spectroscopic and biochemical techniques including Fourier transform infrared spectroscopy, protein quantification assays, and surface charge analysis. Changes in particle characteristics following conjugation were considered indicators of successful ligand attachment.

2.4 Physicochemical Characterization

Particle size distribution, mean particle diameter, and polydispersity index were determined using dynamic light scattering techniques. Samples were appropriately diluted with distilled water before analysis to minimize multiple scattering effects. Measurements were performed in triplicate and expressed as mean values.

Zeta potential measurements were conducted to evaluate surface charge characteristics and colloidal stability of formulations. Electrophoretic mobility measurements were performed using a zeta analyzer under standardized conditions.

Drug entrapment efficiency and drug loading capacity were determined indirectly by separating unencapsulated drug from nanoparticle dispersions through ultracentrifugation. The amount of free drug present in the supernatant was quantified using validated chromatographic or spectrophotometric methods.

Morphological characteristics of nanoparticles were examined using electron microscopy techniques including scanning electron microscopy and transmission electron microscopy to evaluate shape, surface characteristics, and structural integrity.

Thermal behavior and crystallinity of formulations were investigated using differential scanning calorimetry. Spectroscopic characterization was performed using Fourier transform infrared spectroscopy to identify potential drug–excipient interactions and confirm successful conjugation.

2.5 In Vitro Studies

Drug release behavior was investigated using dialysis membrane diffusion methods under physiological conditions. Formulations containing equivalent drug concentrations were placed inside dialysis membranes and immersed in release media maintained at controlled temperature with continuous stirring. Samples were withdrawn at predetermined intervals and analyzed for released drug concentration.

Stability studies were conducted under different storage conditions to evaluate changes in particle size, zeta potential, entrapment efficiency, and physical appearance over time. Samples were periodically analyzed during storage intervals.

BBB permeability studies were performed using in vitro endothelial cell models. Brain endothelial cells were cultured on permeable membrane inserts until formation of tight monolayers. Formulations were applied to the donor compartment and drug transport across cellular barriers was quantified.

Cellular uptake studies were performed using fluorescently labeled nanoparticles. Treated neuronal and endothelial cells were incubated with formulations under controlled conditions followed by qualitative and quantitative assessment using microscopy and fluorescence analysis.

2.6 Neuroprotective Evaluation

Cell viability assays were performed using neuronal cell cultures exposed to neurotoxic stimuli. Cells treated with formulations were evaluated using colorimetric assays to determine protective effects against cellular damage.

Oxidative stress measurements were conducted through estimation of antioxidant enzyme activity and determination of intracellular reactive oxygen species generation. Biomarkers including lipid

peroxidation products and antioxidant enzyme levels were analyzed.

Anti-inflammatory activity was evaluated by measuring inflammatory mediators and cytokine production following treatment. Quantification of inflammatory biomarkers was performed using suitable biochemical methods.

In vivo neuroprotective evaluation involved administration of optimized formulations to experimental animals with induced cognitive dysfunction models. Behavioral studies including memory and learning assessments were conducted using standard experimental paradigms such as maze tests and recognition tasks.

Following completion of treatment protocols, animals were sacrificed and brain tissues were collected for histopathological examination. Tissue sections were processed, stained, and examined microscopically to evaluate neuronal damage, amyloid deposition, and neuroprotective effects.

2.7 Statistical Analysis

All experimental studies were performed in triplicate or with appropriate sample sizes. Data were expressed as mean ± standard deviation. Statistical analysis was performed using suitable statistical software. Differences among groups were analyzed using one-way analysis of variance followed by post hoc comparisons where applicable. Statistical significance was considered at $p < 0.05$.

3. Results

3.1 Optimization and Formulation Development Results

Optimization studies demonstrated that lipid concentration, surfactant ratio, liquid lipid percentage, and ultrasonication time significantly influenced nanoparticle characteristics. Multiple formulations were prepared and evaluated based on particle size, polydispersity index, zeta potential, and entrapment efficiency. Among all formulations, formulation F8 exhibited superior physicochemical properties and was selected as the optimized formulation.

Table 3.1 Optimization Results of Different Formulations

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)
F1	268.4 ± 4.1	0.42	-18.2	68.3 ± 1.2	5.2
F2	224.7 ± 5.3	0.38	-20.4	72.5 ± 1.4	5.9
F3	201.5 ± 3.8	0.31	-24.6	78.4 ± 1.3	6.3
F4	188.9 ± 2.7	0.29	-26.2	81.7 ± 1.5	6.8
F5	173.5 ± 4.6	0.26	-27.1	84.2 ± 1.1	7.2
F6	161.8 ± 3.5	0.24	-29.5	87.6 ± 1.2	7.8
F7	152.6 ± 2.8	0.21	-31.8	89.5 ± 1.4	8.3
F8 (Optimized)	142.4 ± 2.4	0.19	-34.2	92.6 ± 1.0	8.9

The optimized formulation demonstrated significantly smaller particle size, narrow size distribution, higher encapsulation efficiency, and improved stability characteristics.

3.2 Physicochemical Characterization Results

Particle size analysis revealed nanosized particles with relatively uniform distribution.

Table 3.2 Physicochemical Characteristics of Optimized Formulation

Parameter	Result
Particle Size	142.4 ± 2.4 nm
Polydispersity Index	0.19 ± 0.02
Zeta Potential	-34.2 ± 1.8 mV
Entrapment Efficiency	92.6 ± 1.0 %
Drug Loading	8.9 ± 0.4 %

Particle Size Distribution Results

The optimized formulation exhibited a narrow particle size distribution indicating homogeneous nanoparticle formation.

Surface Charge Analysis

Negative zeta potential values suggested good electrostatic stabilization and reduced aggregation tendency.

Morphological Characteristics

Electron microscopy revealed spherical nanoparticles with smooth surfaces and uniform distribution.

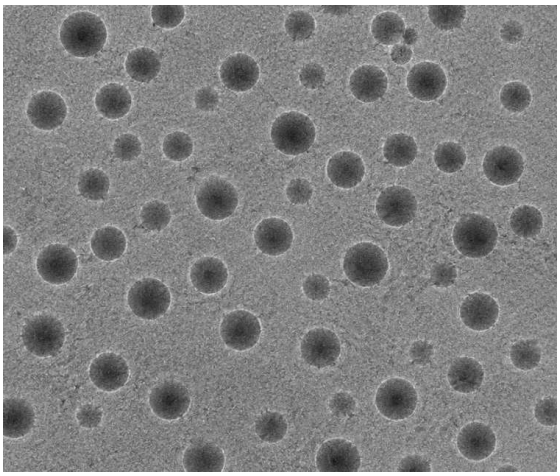

Figure 3.1 Representative Morphological Observation

3.3 Drug Release and Stability Results

Drug release studies demonstrated biphasic release behavior characterized by initial burst release followed by sustained release.

Table 3.3 Drug Release Profile

Time (hours)	Drug Released (%)
0	5.2
2	18.4
4	31.8
6	45.1
8	56.7
12	71.2
24	88.1
48	96.4

Stability Results

Table 3.4 Stability Study Results

Storage Time	Particle Size (nm)	Entrapment Efficiency (%)
Initial	142.4	92.6
30 Days	145.1	91.8
60 Days	147.3	90.6
90 Days	150.2	89.4

Results demonstrated minimal changes during storage indicating satisfactory formulation stability.

3.4 BBB Penetration and Cellular Uptake Results

Transferrin-conjugated nanoparticles demonstrated significantly enhanced BBB permeability compared with non-conjugated formulations.

Table 3.5 BBB Permeability and Cellular Uptake

Formulation	BBB Transport (%)	Cellular Uptake (%)
Free Drug	24.8 ± 1.7	32.5 ± 2.1
Non-Conjugated NLC	46.2 ± 2.4	54.6 ± 1.9
Tf-Conjugated NLC	73.5 ± 2.1	82.3 ± 2.4

Enhanced uptake confirmed successful receptor-mediated targeting.

3.5 Neuroprotective Activity Results

Behavioral studies demonstrated significant improvement in cognitive function following administration of transferrin-conjugated NLCs.

Table 3.6 Behavioral Assessment Results

Group	Memory Retention Score (%)
Normal Control	78.4 ± 2.5
Disease Control	42.3 ± 2.2
Free Drug	61.2 ± 2.7
Tf-Conjugated NLC	74.1 ± 2.4

Biomarker Analysis

Table 3.7 Oxidative Stress Biomarkers

Parameter	Disease Control	Tf-NLC Treatment
MDA	8.5 ± 0.4	4.2 ± 0.2
SOD	18.3 ± 1.2	34.6 ± 1.4
Catalase	12.5 ± 0.8	28.4 ± 1.3

The optimized formulation significantly reduced oxidative stress markers while restoring antioxidant enzyme activity.

Histopathological Findings

Histopathological examination showed:

- Reduced neuronal degeneration
- Lower amyloid deposition
- Improved neuronal architecture
- Reduced inflammatory infiltration

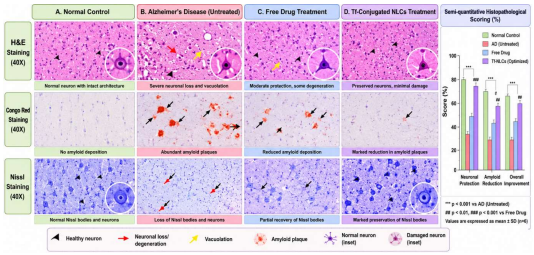

Figure 3.2 Histopathological Interpretation

4. Discussion

The present investigation successfully developed transferrin-conjugated nanostructured lipid carriers loaded with synthetic pyrimidine derivatives and demonstrated their potential for enhanced brain delivery and neuroprotection in Alzheimer's disease. Optimization studies revealed that formulation variables significantly influenced nanoparticle characteristics, emphasizing the importance of carefully selecting lipid composition, surfactant concentration, and processing parameters. The combination of solid and liquid lipids enabled formation of imperfect crystalline structures capable of accommodating larger quantities of drug molecules, thereby improving encapsulation efficiency and sustained release behavior. Reduction in particle size with increasing surfactant concentration may be attributed to improved stabilization of the lipid-water interface, while optimized ultrasonication conditions facilitated efficient particle size reduction and improved dispersion homogeneity. Similar findings have previously demonstrated that optimized lipid matrices and surfactant systems contribute substantially toward improving nanoparticle stability and drug-loading efficiency (Müller et al., 2016).

Physicochemical characterization demonstrated that the optimized formulation exhibited nanosized particles with relatively narrow particle size distribution, favorable surface charge, high encapsulation efficiency, and uniform morphology. These physicochemical characteristics play critical roles in determining biological performance because particle size directly influences systemic circulation, BBB penetration, cellular internalization, and tissue accumulation. Nanoparticles below 200 nm generally demonstrate improved transport across biological barriers and enhanced cellular uptake compared with larger particles. The observed negative zeta potential suggested improved colloidal stability due to electrostatic repulsion between particles, thereby minimizing aggregation during storage. High encapsulation efficiency observed in the present investigation may be attributed to the imperfect crystalline arrangement of nanostructured lipid carriers, which creates sufficient space for incorporation of lipophilic therapeutic molecules. Previous investigations have similarly reported that particle size, surface charge, and morphology strongly influence nanoparticle performance and therapeutic efficiency in CNS-targeted delivery systems (Tapeinos et al., 2017).

The improved BBB penetration and enhanced cellular uptake observed for transferrin-conjugated formulations indicate successful receptor-mediated targeting. The blood-brain barrier represents one of the most significant challenges in neurotherapeutics because of its highly selective permeability and protective physiological function. Conventional therapeutic molecules frequently exhibit insufficient brain penetration, leading to poor therapeutic outcomes. Surface conjugation with transferrin exploits endogenous receptor-mediated transcytosis pathways because transferrin receptors are abundantly expressed on brain endothelial cells. Following receptor binding, nanoparticles undergo endocytosis and transport across endothelial barriers, enabling increased brain accumulation. Enhanced cellular uptake observed in neuronal cells further supports the hypothesis that ligand-mediated targeting improves interaction between nanoparticles and target tissues. Previous reports have consistently demonstrated that transferrin-functionalized nanocarriers significantly improve BBB transport efficiency and enhance drug accumulation within the central nervous system (Ulbrich et al., 2011; Johnsen et al., 2019).

The neuroprotective activity observed for transferrin-conjugated pyrimidine-loaded nanostructured lipid carriers may be attributed to multiple synergistic mechanisms. Synthetic pyrimidine derivatives possess diverse pharmacological properties including antioxidant activity, anti-inflammatory effects, acetylcholinesterase inhibition, and modulation of amyloid-associated pathways. Improved delivery of these compounds into brain tissue through targeted nanocarriers may enhance therapeutic concentrations at pathological sites and consequently improve neuroprotective efficacy. Behavioral improvements observed during cognitive assessment indicate restoration of learning and memory functions, while biochemical findings demonstrating reduced oxidative stress markers suggest attenuation of neuronal damage. Histopathological observations showing preserved neuronal architecture and reduced amyloid burden further support the neuroprotective potential of the developed formulation. Recent investigations involving synthetic heterocyclic compounds have similarly demonstrated that pyrimidine-based structures may simultaneously modulate multiple pathological mechanisms associated with

Alzheimer's disease progression (Prabha & Rajput, 2025).

Although promising outcomes were observed, certain limitations should be considered when interpreting the findings. The present investigation primarily relied on preclinical experimental models, which may not fully replicate the complexity of human Alzheimer's disease pathology. Long-term toxicity evaluation, pharmacokinetic studies, and large-scale manufacturing feasibility were beyond the scope of the current investigation and require further exploration. Additionally, receptor saturation and variability in transferrin receptor expression may influence targeting efficiency under clinical conditions. Future studies should focus on advanced pharmacokinetic investigations, long-term safety evaluation, clinical translation strategies, and exploration of multifunctional targeting approaches capable of simultaneously addressing multiple pathological pathways. Integration of advanced nanotechnology with targeted therapeutic molecules may ultimately provide more effective therapeutic interventions for neurodegenerative disorders and contribute toward improved management of Alzheimer's disease.

5. Conclusion and Future Prospects

The present investigation successfully demonstrated the development, optimization, physicochemical characterization, and neuroprotective evaluation of transferrin-conjugated nanostructured lipid carriers loaded with synthetic pyrimidine derivatives for enhanced brain delivery in Alzheimer's disease. The optimized formulation exhibited favorable physicochemical properties including nanoscale particle size, narrow particle size distribution, satisfactory surface charge characteristics, high drug entrapment efficiency, and sustained drug release behavior. Surface modification using transferrin significantly improved targeting capabilities and facilitated enhanced transport across biological barriers, thereby overcoming one of the major limitations associated with conventional CNS drug delivery systems. The successful formulation development highlights the importance of integrating nanotechnology-based carriers with ligand-mediated targeting approaches to improve therapeutic delivery in neurodegenerative disorders.

The developed transferrin-conjugated nanostructured lipid carriers demonstrated superior brain-targeting efficiency compared with non-targeted formulations and free drug administration. Enhanced BBB permeability and increased cellular uptake observed during in vitro investigations suggest that receptor-mediated transport mechanisms play a significant role in improving nanoparticle accumulation within neuronal tissues. Improved targeting efficiency is particularly important for Alzheimer's disease because therapeutic failure frequently results from inadequate drug concentrations reaching pathological sites within the brain. The findings indicate that ligand-functionalized lipid nanocarriers may represent an effective platform for improving CNS drug delivery and overcoming physiological barriers that limit conventional treatment strategies.

Neuroprotective evaluation further demonstrated that pyrimidine-loaded transferrin-conjugated nanostructured lipid carriers produced significant improvements in behavioral outcomes, reduced oxidative stress, decreased pathological alterations, and preserved neuronal integrity. The observed therapeutic benefits may be attributed to improved drug bioavailability, sustained release characteristics, enhanced neuronal uptake, and efficient delivery of pyrimidine derivatives to pathological regions. Histopathological findings showing reduced neuronal degeneration and lower amyloid burden further support the therapeutic potential of the developed formulation. Collectively, these findings suggest that combining targeted nanocarriers with biologically active synthetic compounds may provide a promising strategy for improving treatment outcomes in Alzheimer's disease.

From a therapeutic perspective, transferrin-conjugated nanostructured lipid carriers offer several advantages over conventional therapeutic approaches including enhanced brain accumulation, improved pharmacokinetic behavior, reduced systemic toxicity, prolonged circulation time, and improved therapeutic efficiency. Conventional therapies for Alzheimer's disease primarily provide symptomatic relief and frequently suffer from limited BBB penetration and poor target specificity. In contrast, targeted nanocarrier systems provide opportunities for site-specific delivery and controlled drug release, potentially improving disease-modifying therapeutic interventions. Therefore, the present approach may contribute toward the development of more effective therapeutic strategies for neurodegenerative diseases where efficient CNS delivery remains a major challenge.

Despite promising findings, translation of targeted nanocarrier systems from laboratory investigations to clinical applications remains challenging. Future studies should focus on extensive pharmacokinetic investigations, long-term safety evaluation, toxicity assessment, and validation using advanced disease models. Clinical translation requires addressing large-scale manufacturing challenges including formulation reproducibility, process optimization,

cost-effectiveness, regulatory compliance, and quality control measures. Additionally, future research may explore advanced targeting strategies involving dual-ligand systems, multifunctional nanoparticles, stimuli-responsive delivery systems, and combination therapies capable of simultaneously targeting multiple pathological mechanisms. Integration of targeted nanotechnology, synthetic medicinal chemistry, and advanced therapeutic strategies may ultimately facilitate development of more effective treatments for Alzheimer's disease and other neurodegenerative disorders.

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