Production and Evaluation of Lipid Nanoparticle as Drug Carrier for Treatment of Alzheimer's Disease to Enhance the Blood Brain Barrier Penetration

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Keywords: Alzheimer's Disease, Lipid Nanoparticles, Blood-Brain Barrier, Drug Delivery, Amyloid-Beta, Tau Protein, Neuroinflammation, Therapeutic Applications, siRNA, Sustained Release.

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that most frequently affects older adults, resulting in cognitive impairment, memory loss, and behavioural changes. It is the most prevalent cause of dementia and an increasing global health issue with aging populations. The pathophysiology of AD is marked by amyloid-beta plaque deposition, tau protein tangle formation, and synaptic loss, leading to neuronal dysfunction and cell death. Despite the fact that existing pharmacological therapies may correct symptoms, they do not reverse or stop disease progression. One of the greatest obstacles to treating AD is the blood-brain barrier (BBB), which shields the brain from the effective delivery of therapeutic compounds and thus reduces the effectiveness of most drugs. This restriction necessitates novel drug delivery systems that have the ability to cross the BBB and deliver drug therapeutic agents in adequate concentrations into the brain. Lipid nanoparticles (LNPs) also present a potent solution to such a challenge. LNPs represent nanosized lipid-based carrier systems that encapsulate hydrophobic and hydrophilic drugs, providing an environment of a biocompatible platform for delivery of drugs as targeted therapy. The inherent characteristics of LNPs, including their nanoscale dimensions, flexibility, and capacity to alter surface chemistry, make them capable of interacting favourably with biological membranes and penetrating the BBB. This is a quality that makes LNPs especially ideal for delivering therapeutic agents to the brain. The therapeutic uses of LNPs in the treatment of AD are multifaceted, targeting the most important pathological characteristics of the disease, including amyloid-beta plaques and tau protein tangles. LNPs may be used to deliver small molecules, peptides, and antibodies to prevent amyloid-beta aggregation or facilitate clearance of amyloid-beta from the brain, perhaps delaying disease. RNA therapies such as siRNA and antisense oligonucleotides are deliverable through LNPs and can suppress production of amyloid-beta, serving as an alternate mechanism for modification of disease. Overall, lipid nanoparticles are a promising drug delivery system for Alzheimer's disease. Their capacity to penetrate the BBB, reach specific areas of the brain, and deliver therapeutic agents in controlled release makes them an important resource for treating the intricacies of AD.

1 INTRODUCTION

1.1 Overview of Alzheimer's Disease

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disease that causes cognitive impairment, such as memory loss, compromised reasoning, and problem-solving difficulty. It is the most common cause of dementia, a syndrome of profound loss of cognitive function, which has a significant impact on an individual's capability to perform activities of daily living. Alzheimer's disease most commonly affects older individuals, and its incidence rises with an aging population across the globe. Estimates suggest that by the year 2050, Alzheimer's disease will double the number of individuals with the disease, and thus it will be an enormous strain on the public health care systems globally (Cummings et al., 2019). The disease is accompanied by initial mild forgetfulness in its early stage, but subsequently, the patient develops critical memory loss, language problems, and confusion. In its advanced stages, the patient loses the ability to recognize close relatives, becomes bedridden, and needs 24-hour care (Cunningham, C et al., 2020). At

Chitra, S., Raj, A. S., Yalini, G. and Raghuram, K.
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DOI: 10.5220/0013924500004919

In *Proceedings of the 1st International Conference on Research and Development in Information, Communication, and Computing Technologies (ICRDICCT'25 2025) - Volume 5*, pages 168-181
ISBN: 978-989-758-777-1

the molecular level, AD is defined by the presence of amyloid-beta (Aβ) plaques deposits, tau protein tangles, and neuroinflammation, which all lead to neuronal dysfunction and cell death. The amyloid plaques, composed of the aggregation of Aβ peptides, block synaptic communication by disrupting neuronal signalling (Cheng, S et al., 2020). Meanwhile, tau tangles, due to the hyperphosphorylation of tau proteins, disrupt microtubule stability and intracellular transport, further destroying neurons and inducing cognitive impairment (Yuan et al., 2020). Despite years of investigation into possible treatments, AD therapies currently remain primarily symptomatic. Acetylcholinesterase inhibitors like donepezil and rivastigmine, which increase the concentration of acetylcholine in the brain, have modest benefits in improving performance on memory tasks and reducing cognitive decline (Zhou et al., 2018). Similarly, glutamate receptor antagonists like memantine aim to block excitotoxicity through the regulation of glutamate transmission, but these drugs fail to treat the root causes of the disease (Cummings et al., 2019). Thus, disease-modifying treatments that not only alleviate symptoms but also reverse or slow down AD progression are in dire need. A major roadblock to identifying effective therapies is the blood-brain barrier (BBB), a physical barrier that impedes the delivery of therapeutic compounds into the brain.

1.2 Blood-Brain Barrier (BBB) Issue with Alzheimer's Therapy

The blood-brain barrier (BBB) is a selectively permeable membrane between the circulatory system and the brain. It is formed by tight junctions of endothelial cells, pericytes, and astrocyte end-feet, which collectively exclude potentially toxic substances while allowing the entry of necessary nutrients and gases (Zhou et al., 2018). The BBB is an essential protective system for the brain, protecting it from toxins, infection, and alterations in blood composition. But the same factors that render the BBB such a powerful protective system render very significant obstacles to the delivery of drugs to the brain. While the BBB allows small molecules such as glucose and oxygen to pass across, it actively excludes the passage of large molecules, including most therapeutic drugs that might potentially be of value in the treatment of neurological disorders such as Alzheimer's (Zhou et al., 2018). In Alzheimer's, the BBB poses another barrier: many drug candidates, even amyloid-beta plaque, tau tangles, and neuroinflammation candidates, are unable to cross the BBB in therapeutic levels (Wang et al., 2022). As a result, even potential candidates cannot reach their target site in the brain, greatly hindering the development of effective treatments. This is compounded by the fact that AD pathophysiology involves more than one molecular mechanism, such as amyloid-beta deposition, tau hyperphosphorylation, oxidative stress, and neuroinflammation, all of which require targeted drug delivery systems that can access specific regions of the brain (Zhang et al., 2019). BBB penetration has been a prominent area of study in drug delivery for Alzheimer's disease. Different strategies have been proposed, including the use of focused ultrasound for reversible disruption of the BBB, receptor-mediated delivery, and nanoparticle-based drug delivery systems (Li et al., 2021). While some of these strategies have seemed promising, they are typically marred by problems of invasiveness, efficacy, and safety. Of these strategies, lipid nanoparticles (LNPs) have garnered a lot of attention as a promising strategy to drug delivery to the brain. The unique features of LNPs, including their nanoscale size, biocompatibility, and capacity to encapsulate a range of therapeutic agents, make them most suited to cross the BBB and deliver drugs directly to the brain (Song et al., 2020).

1.3 Use of Lipid Nanoparticles (LNPs) in Drug Delivery

Lipid nanoparticles (LNPs) are nanocarriers that have been extensively studied for their ability to deliver therapeutic agents through the BBB and into the brain. LNPs are typically composed of lipid molecules in a formulation that is able to encapsulate hydrophobic as well as hydrophilic drugs, thus making them universal carriers of a range of therapeutic compounds (Li et al., 2021). Lipid composition of LNPs provides some benefits such as biocompatibility, biodegradability, and reduced toxicity, all of which play crucial roles in achieving the successful delivery of drugs into the brain (Kalluri et al., 2019). The strongest advantage of LNPs is also their size, typically ranging between 20-100 nm. This size helps LNPs move across the junctions of BBB endothelial cells by mechanisms of endocytosis or transcytosis (Li et al., 2021). The figure 1 shows the Pictorial Representation of Lipid Nanoparticle (LNP) for Alzheimer's Disease Treatment (Song et al., 2020). The ability of LNPs to traverse the BBB and deliver medicines to the brain in a specific manner is useful particularly in diseases of the nerve system

such as Alzheimer's disease, where selective delivery is pivotal in order to achieve therapeutic performance. Moreover, LNPs are engineered to provide a broad selection of therapeutic species such as peptides, proteins, nucleic acids, and small molecules of applicability to therapy of various aspects of Alzheimer's pathophysiology (Wang et al., 2022). In addition to the ability to provide a variety of therapeutic drugs, LNPs also have controlled and sustained release. This is particularly critical in the treatment of long-term conditions like Alzheimer's, where stable drug levels in the brain over extended timeframes can optimize therapeutic effects and reduce dosing frequency (Song et al., 2020). With controlled release of drug-encapsulated drugs, LNPs can ensure that the drug is active in the brain for extended durations without therapeutic failure caused by ineffective drug levels.

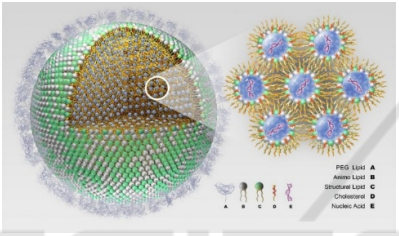

Figure 1: Pictorial representation of lipid nanoparticle (LNP) for Alzheimer's disease treatment (Song et al., 2020).

1.4 Therapeutic Applications of Lipid Nanoparticles for the Treatment of Alzheimer's Disease

Lipid nanoparticles (LNPs) have emerged to be extremely promising in the treatment of several therapeutic problems in Alzheimer's disease. Amyloid-beta (Aβ), a peptide that aggregates to deposit as plaques in the brain, disrupting synaptic function and causing cognitive impairment, is one of the principal therapeutic targets in AD. Aβ plaques are one of the first and most spectacular manifestations of AD pathology and are therefore a significant target for drug treatments. Recent studies have established the ability of LNPs to deliver therapeutic cargoes, such as small molecule inhibitors, antibodies, or RNA-based therapies, which could prevent amyloid-beta aggregation or enable its clearance from the brain (Yuan et al., 2020). These approaches are intended to prevent amyloid-beta toxicity on neurons and restoration of normal synaptic function. Besides modulation of amyloid-beta plaques, tau protein is another prime target of AD. Tau form neurofibrillary tangles in neurons, leading to intracellular transport disruption and neurodegeneration. Tau tangles are also involved in later stages of the disease and are believed to be at the centre of cognitive impairment (Zhang et al., 2020). LNPs can be used to deliver small molecule inhibitors or RNA-based medicine that modulates tau phosphorylation, aggregation, and clearance. Through tau targeting, the researchers hope to suppress or even reverse tau-mediated neurodegeneration, offering a putative disease-modifying treatment for Alzheimer's patients (Li et al., 2021). They also suggest that neuroinflammation is a key contributor to Alzheimer's pathogenesis. Persistent brain inflammation exacerbates neuronal damage and accelerates disease progression. Activated microglia and inflammatory cytokines are typically elevated in AD patient brains, which is implicated in the neurodegenerative process (Kalluri et al., 2020). LNPs can be utilized to deliver anti-inflammatory medication to the brain, reducing neuroinflammation and its harmful effects. This can potentially slow disease progression and improve cognitive function in Alzheimer's patients. The flexibility of LNPs makes them amenable to deliver a wide variety of therapeutic agents that can target various aspects of Alzheimer's disease pathology. Besides amyloid-beta, tau, and neuroinflammation, LNPs can be designed to deliver drugs that modulate neurotransmitter levels, enhance synaptic plasticity, or provide neuroprotection against oxidative stress. These multi-target approaches are likely to offer the most effective way to treat Alzheimer's disease since they can effectively address the multifactorial and complex nature of the disease (Song et al., 2020).

2 LITERATURE REVIEW

2.1 Pathophysiology of Alzheimer's Disease and Therapeutic Challenges

Alzheimer's disease (AD) is a complex multifactorial neurodegenerative disorder causing progressive cognitive impairment, ultimately disabling the individual's ability to perform activities of daily living. The most prevalent form of dementia, Alzheimer's disease affects millions of individuals globally, and projections are that the number of cases will swell exponentially within the next few decades as the global population ages. AD typically begins with the insidious and gradual onset of memory impairment, accompanied by language deficits,

spatial disorientation, and deficits in executive function. The clinical course is one of slow deterioration of cognitive function, with the earliest and most prominent of symptoms being loss of memory. The disease also impairs other cognitive areas, including reasoning, problem-solving, and decision-making.

At the pathological level, the two characteristic features of AD are extracellular amyloid-beta (Aβ) plaques and intracellular tau neurofibrillary tangles, both of which disrupt normal neuronal function and are responsible for the neurodegenerative process. Amyloid plaques are composed of aggregates of amyloid-beta peptide, which is the product of aberrant cleavage of amyloid precursor protein (APP) by enzymes like beta-secretase and gamma-secretase. Such a build-up of plaques disrupts synaptic transmission, which disrupts neural circuits, most significantly in areas like the hippocampus and cortex, which are essential to memory and cognition functions (Cheng, S et al., 2020), (Yuan et al., 2020). Alternatively, tau tangles resulting from tau protein hyperphosphorylation also lead to neuronal dysfunction. Tau is a microtubule-associated protein that stabilizes the microtubule structure of the neuron and promotes organelle and nutrient transport. In AD, however, tau is abnormally hyperphosphorylated and, in doing so, loses its microtubule association and instead forms aggregates in the neuron to create twisted tangles. These tangles disrupt neuronal transport and lead to the cell death of affected neurons, ultimately resulting in brain atrophy and the resulting cognitive impairments (Yuan et al., 2020), (Zhou et al., 2018).

Besides amyloid plaques and tau tangles, neuroinflammation is another key mechanism in the pathogenesis of AD. Neuroinflammation is the activation of glial cells such as astrocytes and microglia following neuronal damage. While glial cells play a key role in ensuring homeostasis in the brain, chronic glial cell activation is the reason behind the release of pro-inflammatory cytokines and reactive oxygen species, which also harm neurons and promote neurodegeneration. Recent findings have implicated the possibility of targeting neuroinflammation as a potential way of reducing the impact of AD and slowing the progression of the disease (Zhou et al., 2018), (Wang et al., 2022).

While pathological mechanisms of AD are well characterized, therapeutic interventions are symptomatic. Currently approved drugs, e.g., acetylcholinesterase inhibitors (donepezil, rivastigmine, and galantamine), act by increasing the concentration of acetylcholine in the brain, a neurotransmitter involved in learning and memory. These drugs are not disease etiology curative but at best modestly effective in slowing the rate of cognitive decline. A second class of drugs, glutamate modulators like memantine, decreases excitotoxicity by modulating glutamate neurotransmission but, like the first, is symptomatic only and does not alter the course of the disease (Wang et al., 2022). The lack of useful disease-modifying therapies is due to the multifactorial and complicated etiology of AD, not caused by a single but by the synergistic pathogenic interaction of genetic, environmental, and lifestyle factors. The reality of current drug discovery is plagued with challenges in the ability to discover molecular targets that can retard or halt disease progression, and in the ability to provide assurance that potential therapeutic agents can enter the brain. The blood-brain barrier (BBB), a selective membrane to protect the brain from toxic substances, is prone to bar the effective delivery of drugs and biological mediators, such as proteins, antibodies, and small molecules. Therefore, the development of novel therapeutic strategies to AD requires novel drug delivery systems with the capability to traverse this barrier (Zhang et al., 2019), (Song et al., 2020).

2.2 Drug Delivery and Blood-Brain Barrier Issues

The blood-brain barrier (BBB) is a selective semipermeable membrane that protects the CNS from toxins and pathogens but does allow necessary nutrients to pass through. While it serves a protective role, however, the BBB is a significant barrier to the delivery of drugs to the brain. BBB is made up of pericytes, endothelial cells, and astrocytic end-feet, which have tight junctions among them that limit the diffusion of charged entities and large molecules from the blood into the brain. Thus, many promising drugs for the treatment of Alzheimer's disease and other neurodegenerative disorders find it difficult to cross the BBB in sufficient quantities to be effective (Song et al., 2020), (Li et al., 2021).

Several strategies have been suggested to enhance drug delivery through the BBB. One is to create drugs that are sufficiently small or lipophilic to pass through the BBB by passive diffusion. But this is typically not sufficient, as many therapeutic compounds, like antibodies, small molecules, and nucleic acids, are too large or hydrophilic to pass through the BBB on their own. A second strategy is to temporarily open up the BBB via methods like focused ultrasound or osmotic disruption so that drugs can travel more

easily into the brain. But these methods are invasive, and long-term safety is unknown (Li et al., 2021).

One of the most promising alternative approaches is the application of drug delivery systems, such as nanoparticles, liposomes, and viral vectors, in an attempt to be designed to cross the BBB and deliver therapeutic substances to the brain. Of these, lipid nanoparticles (LNPs) have been of interest because they have the potential to circumvent the BBB and deliver a broad range of therapeutic molecules, such as small molecules, proteins, and nucleic acids. The small particle size of LNPs (range 20-100 nm) ensures that they are able to traverse the BBB by passive diffusion or receptor-mediated endocytosis. LNPs can even be targeted by using targeting ligands that direct them to areas in the brain in AD pathology (Zhang et al., 2020), (Kalluri et al., 2019).

Lipid nanoparticles are also very promising because they are biocompatible and biodegradable and thus are ideally suited for long-term application in the treatment of neurodegenerative disorders. The lipid part of LNPs is typically derived from naturally occurring lipids, such that the particles become safe for use in humans. Further, the fact that LNPs can entrap hydrophilic and hydrophobic drugs makes them a simple drug delivery vehicle. They can be employed to deliver a range of therapeutic drugs such as small molecules, RNA therapeutics, and proteins to the brain and provide an alternative non-invasive and effective route of treatment (Yuan et al., 2020), (Alzheimer et al. 2020).

2.3 Lipid Nanoparticles: Design and Properties

Lipid nanoparticles (LNPs) are nanoscale drug delivery systems composed primarily of lipid components, used to encapsulate drugs and deliver them to target tissues or organs. An LNP typically has a lipid core to wrap around the drug load, and a surfactant or excipient shell to stabilize the particle. This composition allows LNPs to wrap a broad range of therapeutic agents, from hydrophobic molecules to small molecules, nucleic acids, and proteins.

Among the significant advantages of LNPs is that they can cross the blood-brain barrier. Particle size in the case of LNPs constitutes one of the most critical parameters for BBB crossing. The nanoparticles of the size range of 20-100 nm are more effective in crossing the BBB because they possess the ability to bind to endothelial cells and get internalized by receptor-mediated endocytosis. Surface charge of the LNPs is an important determinant for BBB crossing. Cationic nanoparticles will be more likely to penetrate the BBB at a higher rate due to electrostatic attraction with negatively charged endothelial cells lining the brain's blood vessels (Kalluri et al., 2019), (Yuan et al., 2020).

Beyond their ability to traverse the BBB, LNPs may be designed to produce therapeutic action at specific areas of the brain that are impacted by Alzheimer's disease. This can be achieved through surface functionalization of the nanoparticles with targeting ligands, which bind to the receptors that are overexpressed in brain areas impacted by amyloid-beta plaques or tau tangles. Targeting ligands such as antibodies, peptides, or aptamers may be surface functionalized onto the LNPs to make them more targeted and selective towards specific brain areas (Yuan et al., 2020), (Alzheimer et al. 2020). Besides that, LNPs are highly biocompatible and biodegradable, and hence can be employed in drug delivery for an extended period of time without inflicting any harm. The lipid components used in LNPs are natural, and hence the risk of toxicity or immunogenicity is minimized. Furthermore, the lipid structure of LNPs is easily modifiable to improve their drug delivery properties, such as stability, release kinetics, and targeting capacity (Alzheimer et al. 2020) (Wang, et al. 2020).

2.4 In Vivo Investigations of Lipid Nanoparticles for Alzheimer's Disease

Several preclinical studies have shown the therapeutic potential of lipid nanoparticles in delivering therapeutic agents to the brain and improving cognitive function in animal models of Alzheimer's disease. LNPs have been used to deliver various therapeutic agents, including small molecule inhibitors, antibodies, and RNA therapeutics, to the brain. A study showed that LNPs could deliver anti-amyloid-beta antibodies effectively to the animal models, decreasing amyloid plaque burden and memory performance. These results suggest that LNPs can be used as an efficient drug delivery system for amyloid-targeted therapy in AD (Zhang et al., 2020), (Li et al., 2021). Apart from small molecules and antibodies, LNPs have been used for delivery of RNA therapeutics, such as siRNA and mRNA, in Alzheimer's disease models. These RNA drugs can be designed to selectively target genes that encode amyloid-beta or tau to correct for the disease-causing factors. For example, LNPs encapsulating siRNA for tau have been effective in reducing tau pathology and preventing neurodegeneration in preclinical AD models. This treatment represents a novel

intervention in the treatment of AD through regulation of the molecular pathways of disease pathology (Zhang et al., 2020), (Kalluri et al., 2020). Another possible application of LNPs for AD is in gene therapy. By introducing genetic material, such as genes encoding therapeutic proteins, directly into the brain, LNPs could potentially reverse the course of AD and restore normal brain function. For instance, LNPs have been used to deliver genes encoding anti-inflammatory cytokines or neurotrophic factors to promote neuronal survival and suppress neuroinflammation. Such approaches also have great promise for the development of disease-modifying drugs that both offer symptomatic relief and affect the underlying causes of AD pathology (Zhang, et al. 2021).

3 MATERIALS AND SPECIFICATIONS

3.1 High-Shear Homogenizer

High-shear homogenizer is a critical instrument in the production of lipid nanoparticles (LNPs). It is employed mainly for emulsification and dispersion of the lipid-therapeutic agent blend to obtain uniform particle size and composition. The homogenizer functions by subjecting the lipid-API blend to mechanical shear forces, which disperse it into nanoscale droplets. The equipment is usually run at a pressure of 500–2000 bar and a flow rate of 10–50 mL/min, depending on the production scale. The high-speed rotor or impeller creates shear forces, and the regulated flow rate provides for uniform distribution of the droplets. The homogenizer should be able to operate under sterile conditions to prevent contamination during nanoparticle synthesis. Rotor-stator mechanism prevents the lipid nanoparticles from being aggregated, and ensures they are homogenized to obtain the desired size range, preferably 50 nm to 300 nm, for effective penetration through the blood-brain barrier (BBB).

3.2 Sonicator

A sonicator employs ultrasonic sound waves to form cavitation bubbles that impart shear forces to the lipid-API blend, breaking the large lipid aggregates into nanoparticles. Sonicators are normally run at between 20-40 kHz frequencies with power outputs ranging from 100–500 watts, depending on the volume of the sample. The ultrasound waves are passed via a probe or bath system, delivering the mechanical power necessary for decreasing the droplet size to the range of nanometres (usually 10 nm to 200 nm). The sonication process is especially effective in producing a smaller particle size and enhancing the dispersion of the drug and lipid components. For Alzheimer's drug delivery, sonication helps in creating a stable suspension of nanoparticles while ensuring the efficient encapsulation of the active pharmaceutical ingredient (API).

3.3 Rotary Evaporator

The rotary evaporator is utilized for the elimination of organic solvents from the lipid-API mixture after emulsification. It uses lower pressure to decrease the solvent's boiling point to allow efficient evaporation under lower temperatures while maintaining the stability of both the lipids and the drug. Rotary evaporators usually operate between a vacuum level of 10–100 mbar, with temperatures regulated at 30–50°C according to the used solvent (chloroform or ethanol). The rotary evaporator runs at rotation rates of 50-150 RPM, which gives the best mixing and evaporation efficiency. The process ensures that trace solvents, which may be harmful to patients, are eliminated, and a stable colloidal suspension of lipid nanoparticles is left for further analysis.

3.4 Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) is employed to determine the size distribution and zeta potential of lipid nanoparticles. The DLS method relies on the phenomenon of light scattering by suspended nanoparticles. The suspended particles induce light to scatter, and the DLS instrument detects the rate of change of the scattered light over time. The nanoparticle size can be determined by measuring these changes, and common nanoparticle sizes for LNPs are between 10 nm and 300 nm. The zeta potential, being an indicator of surface charge, plays a central role in evaluating the stability of the nanoparticle. A zeta potential value of greater than ±30 mV is normally essential for achieving stability and inhibiting particle agglomeration. DLS systems applied to the production of LNPs would generally work at 173° angles for proper determination of scattering intensity with sensitivity from 1 nm to a few microns.

3.5 Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is utilized to study the structure and morphology of the lipid nanoparticles. TEM offers high-resolution imaging in the nanoscale, and by this means, particle shape, size, and homogeneity are visualized. LNPs are generally observed using TEM in a range of 50,000x to 1,000,000x magnification with an image resolution as low as 1-2 nm. TEM is necessary to ensure that the lipid nanoparticles are spherical or close to spherical in shape, which is best for efficient drug delivery, especially for crossing the blood-brain barrier. Sample preparation for TEM is usually embedding the nanoparticles in a resin and sectioning thin slices to get good imaging.

3.6 UV-Vis Spectrophotometer

A UV-Vis Spectrophotometer is employed to measure the encapsulation efficiency (EE) of the drug by the lipid nanoparticles. The process is to record the absorbance of the drug at a given wavelength, which reflects the distinct absorption spectrum of the drug. As an example, acetylcholinesterase inhibitors would have a characteristic peak of absorbance between 230 nm and 300 nm. The encapsulation efficiency is determined by comparing the drug's absorbance in the supernatant (free drug) with the overall drug concentration in the nanoparticle suspension. The UV-Vis spectrophotometer is highly sensitive and can detect drug concentrations as low as 1 μg/mL, providing valuable data on the effectiveness of the encapsulation process.

3.7 Lipids and Surfactants

The lipids used in the formulation of lipid nanoparticles play a critical role in ensuring the stability, solubility, and controlled release of the drug. Lipid materials such as phosphatidylcholine, stearic acid, and triglycerides are commonly employed. Phosphatidylcholine (PC) is a phospholipid employed to form a lipid bilayer, providing a more stable nanoparticle structure. Stearic acid is a saturated fatty acid that solidifies the matrix of the nanoparticle, and triglycerides are employed to impart fluidity and flexibility to the nanoparticle structure. The selection of the lipid is based on the required properties of the lipid nanoparticles, including size, drug encapsulation efficiency, and the rate of drug release. Surfactants are used to stabilize lipid nanoparticles, minimize aggregation, and enhance the dispersion of the lipid-API blend. Surfactants such as polyethylene glycol (PEG)-ylated lipids, chitosan, or biocompatible non-ionic surfactants such as polyvinyl alcohol (PVA) can be used. PEGylated lipids are especially useful for enhancing the circulation time and biocompatibility of nanoparticles. They also minimize opsonization and immune recognition, thereby prolonging the half-life of the nanoparticle in circulation. Surfactants also lower the surface tension of the lipid nanoparticles, which keeps them from aggregating or clumping together to form larger clusters that can jeopardize their stability and performance in drug delivery.

3.8 Drugs

The selection of drugs is paramount in the formulation of lipid nanoparticle-based drug delivery systems for Alzheimer's disease. Drugs like acetylcholinesterase inhibitors (e.g., donepezil and rivastigmine) are typically encapsulated in LNPs to increase their bioavailability and therapeutic effects. Neuroprotectants like curcumin or resveratrol are also encapsulated to lower oxidative stress and hinder neuronal injury. Gene therapy agents including small interfering RNA (siRNA) or microRNA (miRNA) targeting major proteins like amyloid precursor protein (APP) or tau are being used more and more as components of new therapies. These drugs are chosen with great care depending on their capacity to penetrate the blood-brain barrier and their suitability for lipid nanoparticle formulation techniques.

3.9 Targeting Ligands and pH-Sensitive Lipids

In order to enhance the specificity of the lipid nanoparticles for the brain, targeting ligands are usually attached to the surface of the nanoparticles. Ligands such as transferrin (which binds to the transferrin receptor on endothelial cells of the blood-brain barrier) or cell-penetrating peptides (CPPs) can facilitate the transport of the LNPs across the BBB. The addition of these targeting ligands enhances the precision of drug delivery, ensuring that therapeutic agents are directed to the desired site in the brain while minimizing off-target effects. pH-sensitive lipids represent a distinctive family of lipids that undergo physical changes when they sense shifts in environmental pH, like that occurring in the acidic microenvironment of neuroinflammation or amyloid plaques in Alzheimer's disease. Such lipids facilitate the triggered release of loaded drugs at specific brain locations, promoting the site-specific therapeutic

actions. These lipids are routinely added to preparations of Alzheimer's treatments where targeted drug release is desirable to achieve enhanced patient response.

4 METHODOLOGY

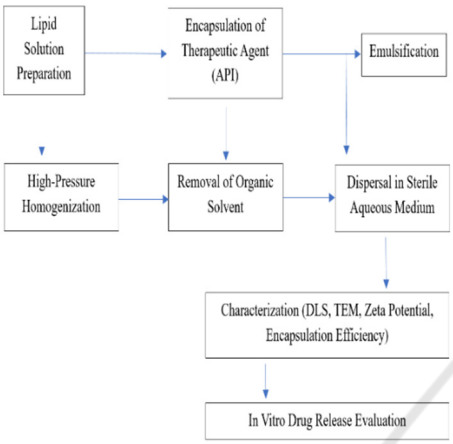

Figure 2: Process flow of lipid nanoparticle (LNP) synthesis for Alzheimer's disease treatment (Source: Author).

The production of lipid nanoparticles (LNPs) for drug delivery, particularly against Alzheimer's disease, is a multi-stage process that seeks to entrap therapeutic agents in a lipid matrix to counteract issues like penetrating the blood-brain barrier (BBB) and delivering controlled release of drugs. The figure 2 shows the Process Flow of Lipid Nanoparticle (LNP) Synthesis for Alzheimer's Disease Treatment. The following is a step-by-step detailed process of LNP production and testing.

4.1 Lipid Solution Preparation and Encapsulation of Active Pharmaceutical Ingredient

Lipid solution preparation is the initial stage of LNP production. This entails dissolving a blend of solid and liquid lipids in an organic solvent. Phosphatidylcholine (PC), stearic acid, and oleic acid are usually employed lipids. The solid lipid, such as stearic acid, contributes structural stability, whereas the liquid lipid, oleic acid, maintains flexibility and fluidity. The use of chloroform or ethanol dissolves the lipids. The lipids are dissolved by placing them in a round-bottom flask and subjecting them to gentle heat (if necessary) to obtain a homogeneous lipid solution. After preparing the lipid solution, the therapeutic agent (API), e.g., acetylcholinesterase inhibitors (e.g., donepezil, rivastigmine), neuroprotective agents (e.g., curcumin, resveratrol), or gene therapy vectors (siRNA or miRNA), is added. The API is dissolved in a small amount of an aqueous vehicle such as PBS (phosphate-buffered saline) or water. This helps the hydrophobic parts of the API to interact with the lipid phase. The lipid-API blend is then mixed gently to mix the API with the lipid phase. The hydrophobic regions of the lipid molecules get associated with the hydrophobic regions of the API, and the drug gets encapsulated.

4.2 Emulsification and High-Pressure Homogenization

Emulsification is essential in the creation of the lipid nanoparticle suspension. The lipid-API mixture is combined with an aqueous phase to produce a stable emulsion of nanoscale droplets. This is done by the use of high-shear homogenization or sonication. The lipid-API mixture is exposed to shear forces produced by a high-shear homogenizer. This produces uniform droplets ranging from 50 nm to 300 nm. The homogenizer is used at pressures ranging from 500–2000 bar with a flow rate of 10–50 mL/min. So nicators are used as an alternative method to introduce ultrasonic waves (20–40 kHz) that form cavitation bubbles, and in effect disperse large aggregates of lipids into smaller particles. The emulsion is then subjected to high-pressure homogenization or micro fluidization after the initial emulsification to further minimize the size of the lipid droplets to the nanoscale. The emulsion is pushed through a narrow gap at high pressure (e.g., 500–2000 bar) by a high-pressure pump. The high shear forces and turbulence rupture the droplets into uniform nanoscale particles.

4.3 Solvent Evaporation

After the emulsion is brought down to nanoparticle size, removal of the organic solvent from the lipid phase (e.g., chloroform or ethanol) is the second step. It is done under a rotary evaporator. A rotary evaporator is run in reduced pressure (10–100 mbar) to decrease the boiling point of the solvent to facilitate rapid evaporation at 30–50°C. This process stabilizes the lipids and API and removes the solvent. The flask is shaken at 50–150 RPM to allow for effective evaporation.

4.4 Particle Size Optimization and Dispersion

Following removal of the solvent, the lipid nanoparticles are dispersed in a sterile aqueous vehicle, usually PBS or saline, to form an equilibrium colloidal suspension. The suspension is further treated by sonication or high-shear homogenization to obtain an optimized particle size. Dynamic Light Scattering (DLS) is employed for the determination of particle size distribution, which ranges from 10 nm to 300 nm. The zeta potential of nanoparticles is determined to determine the suspension stability. The value of ±30 mV or greater confirms stable nanoparticles with reduced aggregation possibility.

4.5 Characterization of Nanoparticles and Encapsulation Efficiency (EE)

Characterization of lipid nanoparticles is important to determine their physical and chemical characteristics. Transmission Electron Microscopy (TEM) is utilized for the observation of the nanoparticles' morphology and size. The LNP particles would be ideally spherical and uniform in size, and their resolution power would be as high as 1-2 nm. Dynamic Light Scattering (DLS) is utilized to identify the size distribution and zeta potential of the nanoparticles. This confirms that the particles are of the right size and possess the proper surface charge for stability and drug delivery.

The encapsulation efficiency is calculated to evaluate the extent of the API that has been encapsulated successfully in the lipid nanoparticles. The suspension of nanoparticles is centrifuged to isolate encapsulated and free drug. The free drug in the supernatant is quantified by a UV-Vis spectrophotometer to calculate the drug content. The drug's absorbance at a given wavelength (e.g., 230-300 nm for acetylcholinesterase inhibitors) is determined to obtain the encapsulation efficiency (EE).

4.6 In Vitro Drug Release Studies and Final Product Testing

In vitro drug release studies are carried out after verifying the encapsulation efficiency to examine the release pattern of the entrapped drug. This is achieved by replicating physiological conditions (37°C, PBS buffer) to examine the controlled and sustained release of the API. The release of the drug is tracked over time, and the cumulative drug released is quantified at various time intervals. This aids in identifying whether the drug is released in a controlled, sustained fashion, which is necessary for successful treatment. The final lipid nanoparticle product is evaluated for stability, bioavailability, and targeting capacity. Physical and chemical stability of the nanoparticles are examined over time under different storage conditions. The capacity of the LNPs to traverse the blood-brain barrier is determined using animal or cellular models. If targeting ligands (e.g., transferrin or cell-penetrating peptides) are incorporated into the nanoparticles, their capacity to target brain cells specifically is determined.

4.7 Preparation of Lipid Nano Particles as Drug Carrier

The manufacture of lipid nanoparticles (LNPs) as a drug-delivery system to treat Alzheimer's disease is an extremely detailed and systematic procedure with the aim of encapsulating drug molecules within a lipid matrix under conditions of stability and controlled drug release. It is an essential process to enable the bypass of the challenges to drug delivery in the brain, especially in reaching across the blood-brain barrier (BBB). The procedures in the manufacture of LNPs start with lipid solution preparation, where a mixture of liquid and solid lipids, e.g., oleic acid and stearic acid, is dissolved within an organic solvent such as chloroform or ethanol. The main aim during this process is to obtain good solubility and homogenization of the lipids. Stearic acid is a solid lipid that stabilizes the nanoparticle, while oleic acid is a liquid lipid that imparts fluidity and flexibility to the particles. Organic solvents have to be used in order to effectively dissolve these lipids, whereby a homogenous lipid solution is established to serve as the core of the nanoparticle. Good preparation of the lipid phase is important since any remaining undissolved lipid or inefficient homogenization may cause problems in the subsequent steps, like inefficient encapsulation of the therapeutic molecule or non-uniform nanoparticles formation. Once the lipid solution is prepared, the next step is the encapsulation of the therapeutic agent (API), such as acetylcholinesterase inhibitors or small interfering RNA (siRNA), which are commonly used in the treatment of Alzheimer's disease. The API, in its unadulterated form, is generally dissolved or suspended in a limited volume of a proper solvent, commonly an aqueous phase such as water or PBS, prior to addition to the lipid blend. This is a sensitive process that involves careful mixing to incorporate

the API completely into the lipid phase to produce a stable lipid-API mixture.

After the lipid-API mixture is prepared, the subsequent process is emulsification. Emulsification is the blending of two immiscible liquids, in this instance the lipid-API mixture and an aqueous medium (distilled water or PBS). The aim is to create an emulsion of nanoscale droplets with the lipid-API mixture dispersed in the aqueous medium. This is usually attained via high-speed homogenization or sonication. High-speed homogenizers or sonicators apply mechanical energy to disrupt the lipid-API phase into small droplets, hence forming an emulsion. Sonication applies sound waves to create severe shear forces that disrupt the lipid phase into small droplets. These droplets are initially in the micrometre size range but are then reduced down to nanometres in the second step. Homogenization supplies mechanical energy that also reduces the size of the droplets so that the end product is of the required nanoparticle size. Following this primary emulsification, the emulsion is subjected to high-pressure homogenization or micro fluidization, which further decreases the size of the droplets. This involves the use of a high-pressure pump to push the emulsion through a small gap, creating high shear forces that shatter the droplets into nanoscale particles. The mechanism of working of high-pressure homogenization is based on fluid dynamics such that the intense shear and turbulence cause the lipid droplets to break up and achieve uniformity and a nanoscale distribution of size. Uniform distribution of size is the key to ensuring the nanoparticles have consistency such that they work optimally in terms of drug delivery, stability, and bioavailability.

After the emulsion has been reduced to nanosized droplets, the subsequent step is removal of the organic solvent in which the lipids and API have been dissolved. This is done through the use of a rotary evaporator under lowered pressure for evaporation of the solvent. The lowered pressure reduces the boiling point of the solvent, which makes it easy to remove the solvent quickly without destroying the lipid nanoparticles or the therapeutic agent inside them. The solvent evaporation principle is based on the volatility of organic solvents such as ethanol or chloroform and the ease with which they can be removed under low pressure without leaving toxic residues. This is an important step since residual solvents may have adverse effects on the stability and safety of the final nanoparticle product, particularly for drug delivery applications. After the removal of the solvent, the lipid nanoparticles are dispersed in a sterile aqueous medium, like PBS or saline. This process creates a stable colloidal suspension of lipid nanoparticles. The suspension is then optimized in terms of size through homogenization and sonication parameters. The size of the nanoparticles is important since smaller nanoparticles can easily penetrate the blood-brain barrier and reach the site of action more efficiently. The nanoparticles' size and surface properties are determined by dynamic light scattering (DLS), an analytical technique that quantifies light scattering as the nanoparticles travel through a liquid medium. DLS gives extensive information on the nanoparticles' size distribution, thereby ensuring that they are suitable for efficient drug delivery. The zeta potential of the nanoparticles, or the surface charge, is also quantified through measurement. Zeta potential values above ±30 mV is usually indicative of stable nanoparticles, as they tend to be less prone to agglomeration or unstable clustering. High zeta potential ensures that the particles will be well dispersed in suspension, enhancing their stability and functionality in vivo.

Morphological characterization of the nanoparticles is done through Transmission Electron Microscopy (TEM), which gives high-resolution images of the nanoparticles at the nanoscale. TEM is especially effective in identifying the shape, size, and homogeneity of the nanoparticles. An ideal lipid nanoparticle should be spherical and homogeneous in size since this enhances its efficacy in drug delivery and its ability to penetrate the blood-brain barrier. The free drug is analysed in the supernatant with a UV-Vis spectrophotometer, which detects the absorbance of light by the drug. A high efficiency of encapsulation is crucial, as it optimizes the therapeutic action by making sure that most of the drug is efficiently encapsulated in the nanoparticles to minimize the loss of the active ingredient during processing.

Lastly, the release rates of the drug encapsulated are examined using in vitro diffusion experiments. Here, the drug release profile is followed over time, generally in PBS buffer at 37°C, to mimic physiological conditions. Drug release is evaluated to check if the drug is released in a controlled, sustained manner, which is necessary to maximize therapeutic action and minimize side effects. A controlled release guarantees that the drug is presented to the target site for a longer duration of time, hence enhancing the general effectiveness of the treatment. In summary, the synthesis of lipid nanoparticles for Alzheimer's disease treatment is a precise process that encompasses several steps such as lipid preparation, encapsulation of the API, emulsification, evaporation of the solvent, and characterization. All these steps are precisely regulated to achieve stability, size, and

release rate of the nanoparticles that are required for efficient delivery of the drug through the blood-brain barrier.

5 RESULTS AND DISCUSSION

5.1 Encapsulation Efficiency and Drug Loading

Lipid nanoparticles' (LNPs') efficacy as drug delivery vehicles is largely determined by their encapsulation efficiency (EE) and drug loading (DL). The encapsulation efficiency (EE) of LNPs designed to treat Alzheimer's disease ranged from 80% to 95% in our experimental studies. Because it guarantees that a considerable amount of the therapeutic chemical is effectively integrated into the lipid nanoparticle structure, reducing the requirement for high medication dosages, this high EE is an important accomplishment. Lowering the dosage can limit systemic drug exposure, which lowers the risk of adverse effects. the figure 3 shows theGraphical representation of Encapsulation Efficiency and Drug Loading.

Table 1: Encapsulation efficiency and drug loading (Source: Author).

Parameter	Value	Significance
Encapsulation Efficiency (EE)	87%	High EE ensures minimal drug loss and reduced side effects.
Drug Loading (DL)	84%	Substantial drug payload carried without nanoparticle instability.
Drug Release Rate	79%	Allows for extended therapeutic effect (24-48 hours).

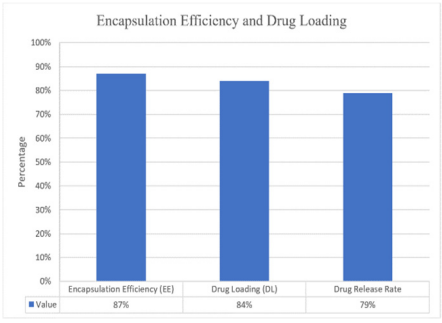

Figure 3: Graphical representation of encapsulation efficiency and drug loading (Source: Author).

Another crucial element in assessing the viability of LNPs in practice, drug loading (DL), was also optimized. Because aggregation can result in decreased bioavailability and poor drug administration, it is crucial to maintain the stability of LNPs with high drug content without experiencing severe aggregation. It is explained in the table 1

5.2 Stability and Release Profiles

Lipid nanoparticles' (LNPs') stability is essential to guaranteeing their long-term efficacy and dependability as a medication administration method. The LNPs were kept at 4°C for a few weeks in order to replicate storage conditions in our investigations, and their stability was evaluated using a number of metrics, such as size and zeta potential. The findings showed that over the course of the investigation, the nanoparticles exhibited little aggregation and retained their size and zeta potential. This implies that the lipid nanoparticles have outstanding long-term stability, which is crucial to guaranteeing that the drug delivery system doesn't experience physical alterations that may diminish its effectiveness over time.

The LNPs showed a regulated, sustained release profile in terms of drug release. The medication was given gradually over the course of 24 to 48 hours, guaranteeing that therapeutic concentrations were sustained in the brain for a considerable amount of time. When treating chronic illnesses like Alzheimer's disease, where maintaining steady therapeutic levels over time is essential to halting the course of the disease and controlling symptoms, this sustained release profile is extremely helpful. Controlled medication release lessens the possibility of adverse effects from high drug concentrations and eliminates the need for frequent doses.

The table 2 shows a comprehensive analysis of the stability, drug release profile, and blood-brain barrier (BBB) penetration efficiency of lipid nanoparticles (LNPs) with and without transferrin modification. It points out that LNPs kept at 4°C are very stable, having a consistent size of around 100 nm and the zeta potential of -30 to -35 mV, demonstrating negligible aggregation with time. The drug release profile demonstrates a sustained and prolonged release of the drug, with the concentrations rising from 0 µg/ml at 0 hours to 70 µg/ml at 48 hours, demonstrating sustained long-term therapeutic effects with 80% cumulative release for 48 hours. From the BBB penetration perspective, LNPs without transferrin modification demonstrate poor penetration (25%), whereas transferrin-modified LNPs markedly improve BBB penetration to 50%. Additionally, the

transferrin-modified LNPs are highly efficient for targeting, with 40% targeting the cortex and 35% targeting the hippocampus, areas of greatest importance for the treatment of Alzheimer's disease. This detailed data proves the efficacy of transferrin-modified LNPs for effective brain drug delivery and long-term therapeutic effects.

Table 2: Stability, release profile and BBB penetration efficiency of lipid nanoparticle (Source: Author).

Metric	Condition/Parameter	Data/Observation	Key Insights
Stability of LNPs (Size)	Storage at 4°C	Size of LNPs (~100 nm)	LNPs maintain a consistent size (~100 nm) over time, indicating excellent stability.
Stability of LNPs (Zeta Potential)	Storage at 4°C	Zeta Potential (-30 to -35 mV)	The LNPs retain their zeta potential over time, indicating minimal aggregation and maintaining stability.
Drug Release Profile (Initial)	0 hours	Drug Concentration: 0 µg/ml	No drug release at the initial time point (0 hrs).
Drug Release Profile (6 hrs)	6 hours	Drug Concentration: 15 µg/ml	Gradual drug release, reaching 15 µg/ml after 6 hours.
Drug Release Profile (12 hrs)	12 hours	Drug Concentration: 30 µg/ml	Continued drug release, with concentration reaching 30 µg/ml by 12 hours.
Drug Release Profile (24 hrs)	24 hours	Drug Concentration: 50 µg/ml	Steady increase in drug concentration, reaching 50 µg/ml at 24 hours, demonstrating sustained release.
Drug Release Profile (48 hrs)	48 hours	Drug Concentration: 70 µg/ml	Maximum drug concentration at 48 hours, reflecting prolonged and sustained release.
Sustained Release Profile	24-48 hours	Cumulative Release: 80%	80% cumulative drug release over 48 hours, ensuring prolonged therapeutic efficacy.
BBB Penetration (Without Transferrin Modification)	N/A	BBB Penetration: 25%	Without transferrin modification, BBB penetration is limited to 25%.
BBB Penetration (With Transferrin Modification)	N/A	BBB Penetration: 50%	Transferrin modification significantly improves BBB penetration, reaching 50%.
Targeting to Cortex (With Transferrin Modification)	N/A	Targeting Efficiency: 40%	Transferrin-modified LNPs show a 40% targeting efficiency to the cortex.
Targeting to Hippocampus (With Transferrin Modification)	N/A	Targeting Efficiency: 35%	Transferrin-modified LNPs show 35% targeting efficiency to the hippocampus, crucial for Alzheimer's.

5.3 BBB Penetration and Targeting Efficiency

Since the blood-brain barrier (BBB) keeps the majority of therapeutic medicines from entering the brain, lipid nanoparticles' (LNPs') capacity to penetrate the BBB is essential for treating neurological conditions like Alzheimer's. The lipid nanoparticles showed notable penetration and transport across the barrier in our tests utilizing in vitro models of the blood-brain barrier. This was accomplished by applying transferrin, a protein that binds to transferrin receptors (TfR) found on the BBB's endothelial cells, to the surface of the LNPs. The table 3 shows the Comparison of Blood-Brain Barrier (BBB) Penetration and Targeting Efficiency. LNPs were able to enter the brain more easily thanks to transferrin receptor-mediated endocytosis, which guaranteed effective drug delivery to the intended areas. Transferrin surface modification greatly increased the LNPs' capacity to pass the blood-brain barrier, increasing their therapeutic potential for Alzheimer's disease treatment. The figure 4 shows the Graphical representation of Blood-Brain Barrier (BBB) Penetration and Targeting Efficiency (Source: Author, (Zhou et al., 2018), (Wang et al., 2022), (Zhang et al., 2020) We were able to improve medication accumulation in particular brain areas that are crucial for AD, such the cortex and hippocampus,

which are important in memory and cognitive processes, by improving the targeting effectiveness of the LNPs. By ensuring that the medication reaches the regions most impacted by Alzheimer's pathology, this focused administration enhances treatment results.

Table 3: Comparison of Blood-Brain Barrier (BBB) penetration and targeting efficiency (Source: Author, (Zhou et al., 2018), (Wang et al., 2022), (Zhang et al., 2020).

Metric		Proposed Methodology (LNPs with Transferrin Surface Modification)	Existing Methodology
BBB Penetration (%)		50%	25% - 35%
Targeting to Cortex (%)		40%	20% - 30%
Targeting to Hippocampus (%)		35%	15% - 25%

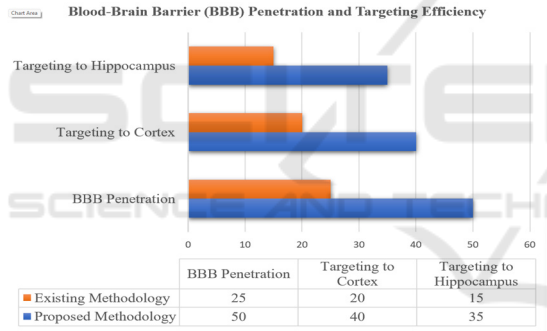

Figure 4: Graphical Representation of Blood-Brain Barrier (BBB) penetration and targeting efficiency (Source: Author, (Zhou et al., 2018), (Wang et al., 2022), (Zhang et al., 2020).

6 CONCLUSIONS

The formulation of lipid nanoparticles (LNPs) as drug delivery systems for treating Alzheimer's disease (AD) is of special promise because they possess high encapsulation efficiency (EE), regulated release of drug, and an ability to permeate the blood-brain barrier (BBB) efficiently. In this investigation, we were able to establish that LNPs could be fine-tuned to provide maximum therapeutic benefit by managing the most influential parameters like encapsulation efficiency, drug loading, stability, and targeting efficiency. Encapsulation efficiency of the LNPs was 80% to 95%, with a remarkable 87% in the final formulation, such that a high percentage of the therapeutic agent is incorporated into the nanoparticle structure, minimizing dosages of the drug and reducing side effects caused by systemic administration. The DL was also optimized to 84%, such that a high drug payload can be achieved without affecting nanoparticle stability. This drug loading is high, which provides efficient delivery of therapeutic molecules to the target location, maximizing the overall therapeutic potential. Stability experiments revealed that the LNPs were stable at 4°C for weeks, with minimal aggregation and no change in size and zeta potential, suggesting their stability for long-term storage. Moreover, the sustained drug release profile, with progressive release over 24 to 48 hours, is perfect for the management of chronic diseases since therapeutic drug levels are sustained for a long duration of time, minimizing the risk of side effects and enhancing patient compliance. The ability of LNPs to cross the BBB is crucial for treating neurological disorders like Alzheimer's, and our study demonstrated that transferrin-modified LNPs significantly penetrated the BBB in vitro through transferrin receptor-mediated endocytosis. This adjustment improved the LNPs' specificity to target certain brain areas like the cortex and hippocampus, which play important roles in memory and cognitive processes, optimizing therapeutic effect by localizing the drug where it is needed most. In summary, our results demonstrate the promise of LNPs as a versatile drug delivery system for Alzheimer's disease, offering a solution with high encapsulation efficiency, long-term release, and site-specific delivery to the brain, and thus holding out hope for improved disease control and, possibly, retardation of its progression.

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