Development and *In-Vivo* Evaluation of Rivastigmine-Loaded PLGA Nanoparticles for Brain Targeting in Alzheimer's disease

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Abstract

Alzheimer disease is a progressive neurodegenerative disease that is marked with memory lapse and cognitive dysfunction. Rivastigmine is a dual acetyl- and butyrylcholinesterase inhibitor and is commonly used in symptomatic therapy, but its clinical activity is severely restricted due to short half-life, low blood-brain barrier permeability and systemic side effects. This current research was to design and test rivastigmine-impregnated poly (lactic-co-glycolic acid) nanoparticles to target the brain better in the case of Alzheimer disease. The emulsion-solvent evaporation method was used to prepare nanoparticles and optimize them in terms of particle size, zeta potential, drug loading and encapsulation efficiency. The optimized formulation was in the form of nanoscale size with narrow distribution and spherical morphology which made it stable and suitable to deliver to the brain. In vitro drug release experiments showed sustained and controlled release behavior as opposed to pure rivastigmine. Pharmacokinetics assessment showed that nanoparticle formulations had higher maximum plasma concentration, longer time to peak plasma concentration, higher area under curve and longer half-life, which is an indication of better bioavailability. Biodistribution studies revealed that there was a high level of accumulation of the drug in brain tissue and the brain targeting index was high in comparison to the pure drug. Animal model behavioral assessment of an Alzheimer disease model established better learning and memory performance of nanoparticles-treated groups over traditional rivastigmine. These results indicate that poly (lactic-co-glycolic acid) nanoparticles are effective brain delivery systems to rivastigmine, which leads to a higher pharmacokinetic effect, targeted biodistribution, and therapeutic effect. The nanoparticulate system developed provides a prospective approach to addressing the shortcomings of the traditional therapy and it can serve as a better method in the management of the disease Alzheimer by means of sustained and targeted delivery of the drugs.

Keywords: Alzheimer's disease, Rivastigmine, PLGA nanoparticles, Brain targeting, Pharmacokinetics, Cognitive performance

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease, which is marked by loss of memory, cognitive and behavioral disturbances. It is the leading cause of dementia and one of the significant health issues facing the world because of the aging population. The pathological characteristics of AD are extracellular deposition of 0 -amyloid plaques, intracellular neurofibrillary tangles of hyperphosphorylated tau protein, synaptic dysfunction, and neuronal loss, especially in the hippocampus and cerebral cortex. These pathological alterations result in abnormal neurotransmission, particularly in the cholinergic system which is important to learning and memory. Although there has been a lot of research, the existing treatment measures are mostly symptomatic in nature and there is no cure to AD (A. T. H. Wu et al., 2021). Rivastigmine, donepezil, and galantamine are examples of cholinesterase inhibitors that are commonly used to enhance cognitive symptoms by raising the concentration of acetylcholine in the synaptic cleft. Rivastigmine is both acetylcholinesterase and butyrylcholinesterase reversible thus it is very beneficial in mild to moderate AD. With that said, traditional formulations of rivastigmine are limited due to a number of factors, such as low plasma half-life (t½), regular dosing schedules, low permeability across the blood-brain barrier (BBB), and side effects at the dosage level, such as nausea, vomiting, and gastrointestinal discomfort (Ekström et al., 2022). These disadvantages decrease compliance by the patient and restrict therapeutic efficacy. There is therefore an urgent requirement of new drug delivery systems that will increase the bioavailability of rivastigmine on the brain and reduce the peripheral exposure and side effects (Asim et al., 2025).

The BBB is a very selective biological barrier created by closely attached endothelial cells, astrocytes and pericytes that shields the brain against harmful substances and selectively permits the passage of vital nutrients. Even though this barrier is important in ensuring brain homeostasis, it limits the accessibility of most therapeutic agents, especially those that are hydrophilic and high-molecular-weight drugs. Only less than 2 percent of small-molecule drugs and nearly none of the macromolecules will permeate the BBB with ease. This renders efficient administration of neurotherapeutics a significant pharmaceutical problem (Daraban et al., 2024). Oxidative stress, inflammation, and partial disruption of the BBB are possible pathological changes in AD, but these changes are not enough to allow the accumulation of drugs in brain tissue with the help of the standard dosage. Hence, sophisticated measures that take advantage of carrier-mediated transportation, receptor-mediated endocytosis, or nanoparticle-based delivery frameworks are needed to surmount this shortcoming (Kim et al., 2024).

Nanotechnology has also become a prospective method of drug delivery to the brain. To increase BBB transport and cellular uptake, nanoparticles can be designed to have the best size, surface charge, and surface functionality. Polymeric nanoparticles among other nanocarriers have some unique strengths such as biocompatibility, biodegradability, targeted release of drugs and enzyme protection of the drug contained in the nanoparticles. Poly(lactic-co-glycolic acid) (PLGA) is among the polymers that have been most widely researched to be used as nanoparticle formulations because it has a good safety profile and in addition it has been approved to be used in humans. The degradation of PLGA into lactic acid and glycolic acid is naturally processed in the body in the Krebs cycle. The physicochemical characteristics of it can be modified by changing the ratio of lactic to glycolic acid and molecular weight enabling a fine tuning of drug release kinetics and particle stability (Ghosh et al., 2025; Naqvi et al., 2020).

PLGA nanoparticles have the capacity to entrap hydrophilic and lipophilic drugs and release them on long-term basis. In the case of brain delivery nanoparticles between 100-200 nm are regarded as the best as they can escape the clearance of the reticuloendothelial system and also transcytose the brain across the BBB, either through endocytosis or adsorptive-mediated transcytosis. Moreover, addition of surface stabilizers or targeting ligands to PLGA nanoparticles can be used to increase further brain uptake. The characteristics of the PLGA nanoparticles render it an appealing carrier of rivastigmine in the treatment of AD (Zhi et al., 2021). The process of brain targeting with the help of PLGA nanoparticles is connected to various pathways. To begin with, nanoparticles ensure that rivastigmine is not degraded during the systemic circulation, thus increasing its t½. Second, the size of the nanoparticles and their surface properties allow the interaction of PLGA nanoparticles with endothelial cells of the BBB and their internalization via endocytic processes. Third, pathological processes like AD may elevate the BBB permeability due to enhanced oxidative stress and neuroinflammation, hence preferring the accumulation of nanoparticles in the brain tissue. After internalization, nanoparticles release rivastigmine in a regulated way, which ensures therapeutic concentrations in the target site

during long periods of time. This controlled delivery does not only increase the therapeutic effect but also decreases the dose administration and the systemic toxicity (A. Cunha et al., 2021).

Besides better pharmacokinetics, delivery via nanoparticles can potentially increase the pharmacodynamic response of rivastigmine. Higher levels of inhibition of cholinesterase enzymes can be induced locally by raising the concentration of drugs in the brain thus resulting in enhanced cholinergic neurotransmission. This may be converted into improved cognitive performance, retention and behavioral results of AD models. Furthermore, systems based on nanoparticles can provide a possibility of simultaneous delivery of two or more agents or imageries, which creates a new opportunity of theranostic use in the neurodegenerative conditions (Rompicherla et al., 2021). Although these are such promising benefits, rivastigmine is to be formulated into PLGA nanoparticles, and optimization of the process must be done to obtain desirable particle size, drug loading, and release properties. The concentration of the polymer, the type of solvent, the concentration of the emulsifier, and the manner of preparation among other parameters has a great effect on the properties of the nanoparticles. Moreover, in vivo testing has to be done fully in order to determine the pharmacokinetic profile, biodistribution, and pharmacological activity of the formulation that is developed. The AD animal models offer useful resources to evaluate the memory capacity, learning capability, and biochemical indicators after treatment. These researches will be necessary to prove the possibility of nanoparticle-based delivery systems to overcome the drawbacks of conventional rivastigmine treatment (S. Cunha et al., 2020).

Thus, the current research paper aims at developing rivastigmine-conjugated PLGA nanoparticles and testing its capability to target the brain in the case of AD. The paper will create a stable and optimized nanoparticulate system, describe the physicochemical characteristics of the system, and examine the performance of the nanoparticulate system in vivo, including pharmacokinetics, biodistribution, and therapeutic efficacy. This research aims to offer a logical ground on how to enhance the delivery of rivastigmine to the brain and increase its clinical usefulness in the management of AD by combining formulation science with biological assessment. This research could be valuable in the development of nanoparticles in treatment of neurodegenerative disorders and contribute to new knowledge on the application of targeted delivery of drugs across the BBB (Imam et al., 2024).

2. Mechanism of Brain Targeting in Alzheimer's disease

The BBB is a very specialized interface of endothelial cells joining the tight junctions, astrocytes and pericytes, which are collectively involved in the regulation of the flow of substances between the systemic circulation and the central nervous system. In physiological conditions, most therapeutic agents are limited to enter the brain through the BBB, especially the hydrophilic and high molecular weight ones; this limits their bioavailability to the brain (Lochhead et al., 2020). However, in AD, BBB integrity can be impaired by pathological changes in the form of chronic neuroinflammation, oxidative stress, and amyloid-B-deposition. All these pathological processes contribute to the partial destruction of tight junction proteins and the endothelial layer permeability. Though this perturbation is not dramatic enough to warrant free entry of drugs, it forms a good platform on which nanoparticle-based delivery systems can have increased brain uptake relative to traditional dosage forms (D. Wu et al., 2023).

Figure 1 illustrates the cholinergic synapse and the mechanism of action of anticholinesterase drugs used in Alzheimer's disease therapy. In the presynaptic neuron, acetyl-CoA derived from mitochondria combines with choline via choline acetyltransferase to form acetylcholine, which is stored in synaptic vesicles and released into the synaptic cleft. Acetylcholine binds to receptors on the postsynaptic neuron to mediate neurotransmission. Acetylcholinesterase normally hydrolyzes acetylcholine into choline and acetate, terminating the signal. As shown in Figure 1, drugs such as rivastigmine, donepezil, and galantamine inhibit acetylcholinesterase, thereby increasing synaptic acetylcholine levels and improving cognitive function (Chen et al., 2022).

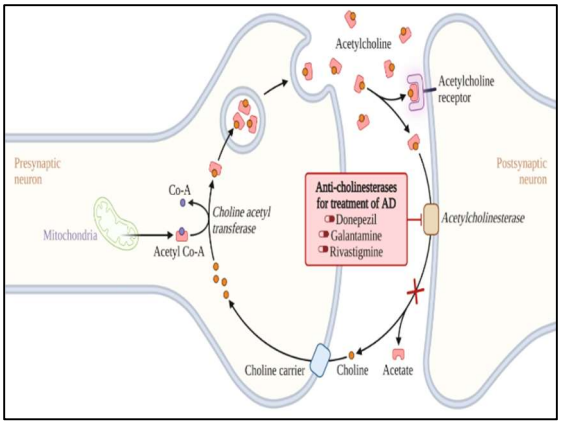

Figure 1: Cholinergic synapse and mechanism of action of anticholinesterase drugs in *Alzheimer's disease; Acetylcholine synthesis, release, receptor binding, enzymatic degradation, and inhibition of acetylcholinesterase by rivastigmine, donepezil, and galantamine to enhance cholinergic neurotransmission.*

Nanoparticles of an optimal size have the ability to interact with BBB endothelial cells and be internalized through endocytic pathways. By this process, they are able to circumvent the paracellular tight junction barriers and cross the BBB using transcellular means of transport. Activated microglia and inflammatory mediators in the context of AD also promote the accumulation of nanoparticles in the brain tissue by modulating the vascular permeability and endothelial uptake. Therefore, BBB dysfunction associated with disease is a valuable physiological foundation to improve the provision of nanoparticulate drug systems to the brain (Asimakidou et al., 2024). Polymeric nanoparticles, including the systems based on PLGA, can cross the BBB by the mechanisms of receptor-mediated and adsorptive-mediated transcytosis. Their nanoscales and surface characteristics enable them to react with components of cell membranes of the endothelial cells. Nanoparticles whose surface is positively charged or whose surface has been modified with a negative charge may bind to negatively charged membrane domains and be internalized by the cell using endocytosis mediated by clathrin or caveolae. After their internalization, the nanoparticles are carried across the endothelial cells in vesicular compartments and discharged on the brain side of the BBB (W. Zhang et al., 2021).

Besides passive transport, nanoparticles may be functionalized to surface by means of the use of specific ligands, which can lead to receptor-mediated uptake. These ligands attach to receptors of high expression levels to the BBB endothelial cell including transferrin or low-density lipoproteins receptor, which facilitates increased nanoparticle uptake into brain tissue. Even though the passive PLGA nanoparticles depend on physicochemical interactions as a major mechanism to cross the BBB, the sustained release properties are useful in the continued release of the drug that is encapsulated in the nanoparticles within the brain environment (Baghirov, 2025). After transcytosis, nanoparticles can be found in the brain interstitial fluid and slowly empty the loaded drug. This slow absorption enables rivastigmine to sustain therapeutic levels in the areas of the brain over a long period of time. Nanoparticle-based delivery enhances retention of drugs in the site of action by reducing the reabsorption of the drug into the systemic circulation. The effect of increased cellular uptake, transcytosis, and prolonged release of drugs together plays a crucial role in AD therapy through brain targeting of the PLGA nanoparticles (Ezzaki et al., 2025).

Therapeutic success of rivastigmine delivery to the brain is not only determined by the ability to traverse the BBB but also sufficient levels of the drug in the neuronal locations linked to cognitive performance. The neurons in the hippocampus and cortex are cholinergic cells, which are badly affected in AD leading to the low levels of acetylcholine and a decreased neurotransmission. Rivastigmine provides its effects by blocking acetylcholinesterase and butyrylcholinesterase, and raising the concentration of synaptic acetylcholine. Nevertheless, traditional preparations cause the quick rise and fall of the plasma drug concentrations, which result in non-optimal and temporary actions in the brain (Mishra & Yadav, 2025). Nanoparticles made of PLGA offer sustained and controlled release of rivastigmine, which prolongs the exposure of cholinergic neurons to the drug. This prolonged release enhances pharmacodynamic response, which ensures constant enzyme inhibition and stabilization of neurotransmitter concentration. Additionally, nanoparticle-based systems minimize systemic distribution of the drug and exposure of the side effects to peripheral tissues, which lowers the adverse effect of traditional oral therapy (Abbas, 2021).

Neuroinflammation and oxidative stress are also added to the susceptibility of neurons in AD pathology. A possible way in which nanoparticle-mediated delivery of rivastigmine can indirectly regulate these pathological processes is by enhancing neuronal survival and synaptic activity via an increase in cholinergic transmission. The effect of the long retention of the drug in the brain is also helpful in achieving better therapeutic outcome in relation to

memory storage and cognitive functioning. Therefore, the synergistic action of BBB transport, transcytosis mediated by nanoparticles, and continuous drug delivery is the underlying basis of the brain targeting of AD with nano-particulate systems made using PLGA (Youssef et al., 2025).

3. Materials and Methods

3.1. Materials

Rivastigmine hydrogen tartrate was purchased at Sigma-Aldrich (Merck Life science Pvt. Ltd., Gurugram, Haryana) (Invoice No. ML/NCR/RT/2025/117). PLGA; 50:50, 30,000 60,000) was purchased at Evonik Industries India Pvt. Ltd., New Delhi (Invoice No. EV/NCR/PLGA/2025/089). Polyvinyl alcohol (PVA; 87 -89 percent hydrolyzed) as a stabilizer was bought at Sisco Research Laboratories (SRL Pvt. Ltd., Faridabad, Haryana) (Invoice No. SRL/NCR/PVA/2025/245). Rankem Chemicals, New Delhi (Invoice No. RK/NCR/SOL/2025/332) provided dichloromethane and acetone (analytical grade). The phosphate buffer saline (PBS) and other analysis reagents were purchased with the HiMedia Laboratories Pvt. Ltd., Delhi (Invoice No. HM/NCR/REAG/2025/406). Chemicals were of analytical grade and were not further purified. The preparation of the double-distilled water was done in-house and consumable during the experimental work.

3.2. Preparation of Rivastigmine-Loaded PLGA Nanoparticles

The emulsion-solvent evaporation method was used in preparing Rivastigmine-loaded PLGA nanoparticles. In short, an organic phase was prepared by dissolving a weighed amount of PLGA in dichloromethane and rivastigmine was subsequently added to the solution, through constant stirring. This organic phase was slowly added to an aqueous phase that had PVA as a stabilizing agent and was homogenized using high speed to achieve a fine emulsion of oil-in-water. Probe sonication was also performed to the emulsion with the aim of decreasing the size of droplets and enhancing the homogeneity of nanoparticles (Joshi et al., 2010). For this purpose, the organic solvent was eliminated through constant magnetic stirring at the ambient temperature, and this resulted in the creation of solidified PLGA nanoparticles entraping rivastigmine. The nanoparticle suspension was centrifuged, washed with distilled water to eliminate the excess stabilizer and drug not encased by the nanoparticle and lastly lyophilized to obtain the dry nanoparticle powder. The variables of the formulation were optimized to obtain desirable particle size, drug entrapment efficiency and release properties (Alkholief et al., 2022). The critical formulation parameters that included concentration of PLGA, ratio of drugs and polymer, concentration of PVA, homogenization rate and duration of sonication were systematically manipulated. The influence of each variable on the nanoparticle size distribution, zeta potential and drug loading was assessed. On the basis of these findings, an optimized formulation was chosen to be further characterized by physicochemical and in vivo assessment (Alonso et al., 2022).

3.3. Physicochemical Characterization

The physicochemical characteristics of rivastigmine-loaded PLGA nanoparticles were determined based on the particle size, polydispersity index (PDI), zeta potential, morphology, drug loading, and entrapment efficiency. Dynamic light scattering (DLS) was used to determine the mean particle size, size distribution and the zeta potential of the nanoparticles after proper dilution with distilled water. The PDI value was employed as a measure of uniformity of the particle sizes, and the lower the values, the narrower is the size distribution which is good in brain delivery. To determine surface charge and colloidal stability of the nanoparticle dispersion, Zeta potential was determined (Priya et al., 2023). The morphology and shape of the prepared nanoparticles were analyzed by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Before analysis, samples were stuck on aluminum stubs, sputter-coated with a thin layer of gold in case of SEM, and TEM samples were prepared by dropping nanoparticle suspension on a carbon-coated copper grid and letting it air-dry. Drug loading and entrapment efficiency was established by dissolve a known amount of nanoparticles in an appropriate organic solvent to release rivastigmine and quantified a known amount of rivastigmine using a validated analytical technique. The standard mathematical equations were used to calculate the percentage drug loading and entrapment efficiency (Kaur et al., 2011).

3.4. In Vitro Drug Release Study

The diffusion of rivastigmine into a dialysis bag was used to assess the in vitro release of rivastigamine by PLGA nanoparticles. A certain amount of rivastigmine-loaded nanoparticles corresponding to a set dose of medication was weighed correctly and

suspended in PBS (pH 7.4) and put in a dialysis membrane that was pre-soaked. A dialysis bag was placed in a beaker with the release medium, and it was held in 37 ± 0.5 o C on a continuous stirring to replicate the physiological condition. Aliquots of the release medium were sampled at specified time points and an equal amount of fresh buffer added to the sample to keep sink conditions constant (Pavaloiu et al., 2021). The amount of rivastigmine in the samples was determined by a proven analytical procedure and the cumulative percent of the drug being released was determined. The release data were plotted against the different kinetic models to determine the mechanism of the drug release through the nanoparticles, the models used was; zero-order, first-order, Higuchi, and Korsmeyer Peppas models. The values of correlation coefficient were used to identify the best-fitting model. This kinetic study aided in the determination of drug release as diffusion-controlled, erosion-controlled or anomalous transport of the drug out of the PLGA matrix (Ismail et al., 2013).

3.5. In Vivo Experimental Design

The in vivo study was conducted on healthy adult Wistar rats (200250 g) of either sex due to their appropriateness in pharmacokinetic and behavioral analysis in the models of AD. Animals were kept in the normal laboratory conditions (25 ± 2 o C, 55 ± 5 relative humidity and 12 h of light-dark cycle) and allowed to eat freely standard pellet diet and water. All experiments were carried out as per the requirements of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India (Vore et al., 2024). The study was initiated only after the experimental protocol had been pre-approved by the Institutional Animal Ethics Committee Jamia Hamdard (IAEC/2025/PHARM/07).

The animals were randomly divided into five groups, each containing six rats (n = 6):

- **Group I (Normal Control):** Received normal saline and served as the healthy control group.
- **Group II (Disease Control):** Received AD induction agent without treatment.
- **Group III (Standard Treatment):** Received pure rivastigmine solution at the therapeutic dose.
- **Group IV (Blank Nanoparticles):** Received drug-free PLGA nanoparticles to assess carrier safety.
- **Group V (Test Group):** Received rivastigmine-loaded PLGA nanoparticles equivalent to the dose administered in Group III.

The administration of all formulations was done through the intraperitoneal route daily based on the set of the study. Behavioral tests and biological sampling were conducted at a specific time in order to compare the pharmacokinetic behavior and therapeutic efficacy across various treatment groups (Al Shoyaib et al., 2020).

3.6. Pharmacokinetic and Biodistribution Studies

The effectiveness of the developed PLGA nanoparticle formulation was determined by pharmacokinetic and biodistribution studies to assess the systemic circulation profile and brain-targeting efficiency of rivastigmine after it was administered as a solution of the nanoparticle formulation. Animal blood samples were taken at a set time interval following the administration of the drug by the retro-orbital plexus under light anesthesia. The samples were moved to the heparinized tubes and centrifuged at 4000 rpm with 10 min to separate the plasma which was kept at -20 C until analysis (Yousfan et al., 2024). In biodistribution research, animals were euthanized at chosen time points and then the brain tissues were excised, rinsed in ice-cold normal saline, dried on a blot and weighed. A tissue homogenizer was used to homogenize the collected brain samples in phosphate buffer (pH 7.4). Centrifugation of homogenate was conducted and supernatant was taken to estimate the drug (D. Zhang et al., 2024). The rivastigmine concentration of the plasma and homogenate of the brain was assessed by an established analytic method like the high-performance liquid chromatography. The stationary and the mobile phases were selected as appropriate and the chromatographic separation was done under optimized conditions. Pharmacokinetic characteristics such as maximum plasma concentration (Cmax), time to maximum concentration (Tmax), area under the curve (AUC) of concentration versus time, and t½ were determined. The nanoparticle formulation was evaluated based on brain-to-plasma drug concentration ratios that assessed brain-targeting efficiency of the drug (Imam et al., 2024).

4. Results

4.1. Nanoparticle Characterization

The produced rivastigmine-loaded PLGA nanoparticles were tested on the mean particle size, surface charge, and morphological characteristics to ascertain their appropriateness in the delivery of rivastigmine to the brain. The analysis of the DLS showed that the size of the nanosized particles has a small size distribution, which is a sign that the formulation is uniform and that the particles are well dispersed. The average particle size was kept in a range of optimal particle sizes in transporting BBB. The values of Zeta potential were reasonably negative indicating that the dispersion was electrostatically stabilized and the aggregation of particles was minimized. The morphological analysis with the help of SEM indicated that the nanoparticles were mainly spherical and with smooth surfaces and no cracks or drug crystals could be seen on the surface. The fact that there were no surface irregularities ensured that rivastigmine was efficiently encapsulated in the PLGA matrix. Small differences in batch sizes can be explained by the fact that homogenization and evaporation rates of the solvents during preparation can be different. In general, the integrated outputs of the particle size analysis, the surface charge analysis, and SEM imaging proved the effective development of stable and homogeneous nanoparticles applicable in the long-term brain targeting. It is hoped that these physicochemical properties will lead to better circulation time, reduced rapid clearance, and better therapeutic performance of rivastigmine in the treatment of the AD.

Table 1: Characterization of Rivastigmine-Loaded PLGA Nanoparticles

Batch	Particle size (nm)	Zeta potential (mV	SEM size (nm	PDI
F1	165.2 ± 2.1	−20.4 ± 1.2	150.6 ± 1.8	0.22
F2	168.4 ± 2.3	−21.3 ± 1.1	152.1 ± 2.0	0.21
F3	170.1 ± 2.5	−22.0 ± 1.3	154.2 ± 2.2	0.23
F4	166.8 ± 2.0	−20.9 ± 1.0	151.4 ± 1.7	0.20
F5	169.5 ± 2.4	−21.7 ± 1.2	153.6 ± 2.1	0.22

SEM: Standard error of mean; Mean particle size = 168.0 ± 2.26 nm; Mean zeta potential = −21.26 ± 1.16 mV; Mean SEM size = 152.38 ± 1.96 nm.

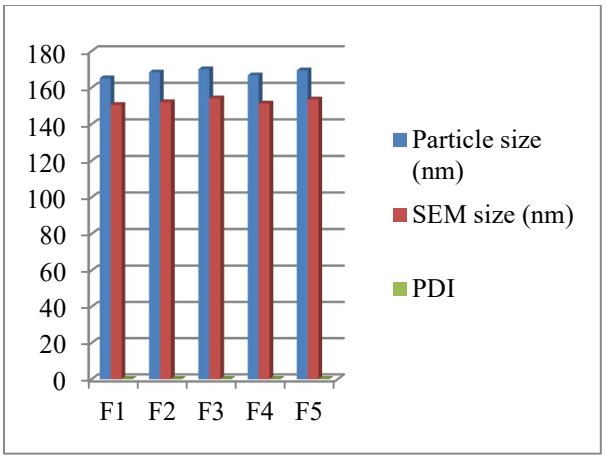

Figure 2: Characterization of Rivastigmine-Loaded PLGA Nanoparticles

4.2. Drug Loading and Encapsulation Efficiency

Quantitative analysis of rivastigmine-loaded PLGA nanoparticles indicated that the drug loading procedure and the encapsulation efficiency were satisfactory that validated the appropriateness of the formulation technique. The loading of the drug was a little different in different batches because of the variation in the concentration of polymer and the drug-polymer ratio, and the encapsulation efficiency was a regular and reproducible trend. The addition of more and more PLGA enhanced the encapsulation efficiency, as the polymeric matrix was formed, became thicker, and reduced diffusion of the drug into the external aqueous phase during the emulsification process. Nevertheless, polymer concentration was too high causing high viscosity, which caused slightly lower drug loading. Optimization experiments showed that moderate stabilizer concentration and an equal amount of drug and polymer were important in attaining maximum entrapment without disturbing particle size and stability. The optimized formulation had a high encapsulation efficiency and acceptable drug loading so that the therapeutic payload per unit mass of nanoparticles is satisfactory. These findings indicate that the formulation variables are very important in regulating drug distribution within the polymeric matrix and directly affect the work of the nanoparticulate system. In general, the optimized rivastigmine-loaded PLGA nanoparticles represented an effective carrier system that could deliver an adequate quantity of drug to be released in a sustained manner and brain targeting during the treatment of the AD.

Table 2: Drug Loading and Encapsulation Efficiency of Different Formulations

Batch	Drug loading (%)	Encapsulation efficiency (%)	Polymer (mg)	Drug (mg)
F1	6.8 ± 0.3	72.4 ± 1.8	100	10
F2	7.3 ± 0.4	75.9 ± 2.0	120	10
F3	7.9 ± 0.5	82.1 ± 2.3	150	10
F4	7.5 ± 0.4	79.6 ± 2.1	140	10
F5	8.2 ± 0.5	85.4 ± 2.5	160	10

SEM: Standard error of mean; Mean drug loading = 7.54 ± 0.42%; Mean encapsulation efficiency = 79.08 ± 2.14%.

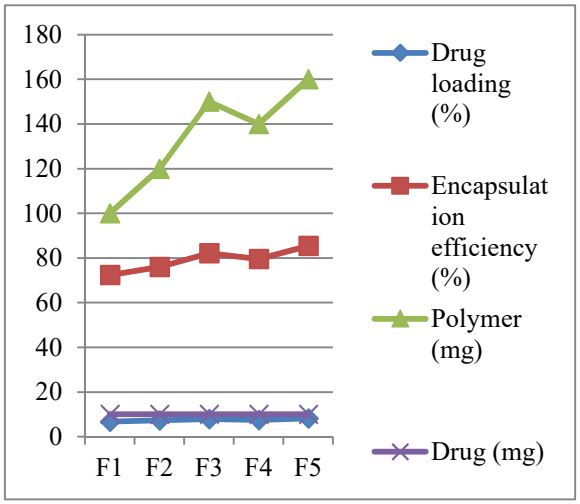

Figure 3: Drug Loading and Encapsulation Efficiency of Different Formulations

4.3. In Vitro Drug Release Profile

The drug release behavior of rivastigmine-loaded PLGA nanoparticle formulations (F1 -F5) in phosphate buffer (pH 7.4) was compared with the drug release behavior of a pure drug suspension. Enhanced dissolution behavior owing to nanosizing and polymeric encapsulation was observed because all nanoparticle formulations exhibited a much greater and more prolonged cumulative drug release in comparison to the pure drug. Of all the formulations, F3 had the highest drug release of 74.3=2.10/percent in 24 h, which could be explained by its optimum polymer to drug ratio and homogeneous particle size that improved diffusion and polymer erosion. Conversely, the pure drug suspension only emitted 38.6 + 1.42 percent in the identical time, which means that it is not aqueously soluble. The diffusion-controlled release of drugs was indicated by the release pattern of nanoparticle formulations being followed by Higuchi kinetics. These results indicate that the optimized rivastigmine-PLGA nanoparticles are effective in the dissolution of drugs and controlled release that is likely to enhance therapeutic efficacy and reduce the frequency of dosage as compared to the traditional dosage form.

Table 3: *In-Vitro* Cumulative Drug Release of Rivastigmine from PLGA Nanoparticles

Formulation	Drug: Polymer Ratio	% Drug Release (12 h)	% Drug Release (24 h)	Mean Value
Pure Drug	–	22.4 ± 1.15	38.6 ± 1.42	30.5
F1	1 : 1	48.7 ± 1.63	65.2 ± 1.84	56.95
F2	1 : 2	53.6 ± 1.71	70.4 ± 1.92	62.0
F3	1 : 3	57.6 ± 1.86	74.3 ± 2.10	65.95
F4	1 : 4	55.2 ± 1.78	72.1 ± 1.96	63.65
F5	2 : 1	50.4 ± 1.69	68.5 ± 1.88	59.45

Values are expressed as mean ± SEM (n = 3).

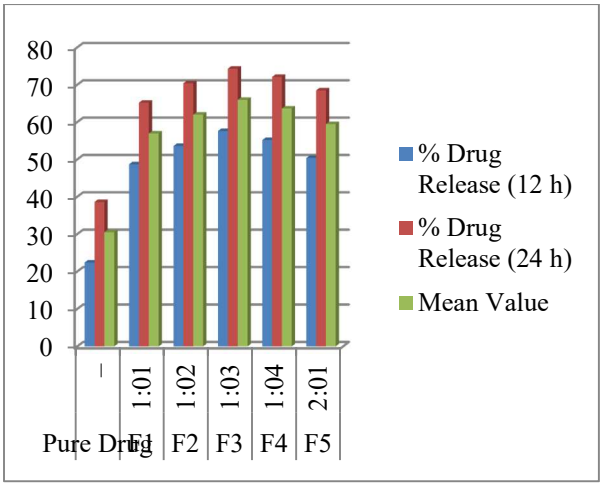

Figure 4: *In-Vitro* Cumulative Drug Release of Rivastigmine from PLGA Nanoparticles

4.4. Pharmacokinetic Parameters

To determine the changes in the systemic exposure and absorption, the pharmacokinetic parameters of

the rivastigmine-loaded PLGA nanoparticles were compared with those of the pure drug. Nanoparticle formulations exhibited increased Cmax and delayed Tmax relative to the pure drug and this represents better absorption and controlled release characteristics. Nanoparticle formulations demonstrated a significantly greater increase in AUC, which confirmed an increase in bioavailability and an increase in the time of drug presence in the systemic circulation. The t½ of rivastigmine was also prolonged in all the nanoparticle-treated groups indicating less elimination and prolonged release of the drug at the polymeric matrix. Conversely, pure drug was well absorbed and cleared, and its exposure and duration of action were lower. In general, the results indicate that the nanoparticles delivery method leads to the enhancement of the pharmacokinetic characteristics of rivastigmine and can decrease the dose frequency.

Table 4: Pharmacokinetic Parameters of Rivastigmine Formulations

Parameter	Pure Drug	F1-NPs	F2-NPs	F3-NPs	F4-NPs	F5-NPs
Cmax (ng/mL)	85.4 ± 3.2	112.8 ± 4.1	125.4 ± 4.5	138.6 ± 5.0	132.7 ± 4.8	118.9 ± 4.3
Tmax (h)	1.0 ± 0.1	2.0 ± 0.2	2.5 ± 0.2	3.0 ± 0.3	2.8 ± 0.2	2.2 ± 0.2
AUC (ng·h/mL)	320.6 ± 12.4	465.3 ± 15.6	512.7 ± 18.2	578.9 ± 20.4	545.6 ± 19.1	488.4 ± 16.8
t½ (h)	2.1 ± 0.2	3.6 ± 0.3	4.1 ± 0.3	4.8 ± 0.4	4.5 ± 0.3	3.9 ± 0.3

Values are expressed as Mean ± SEM (n = 6).

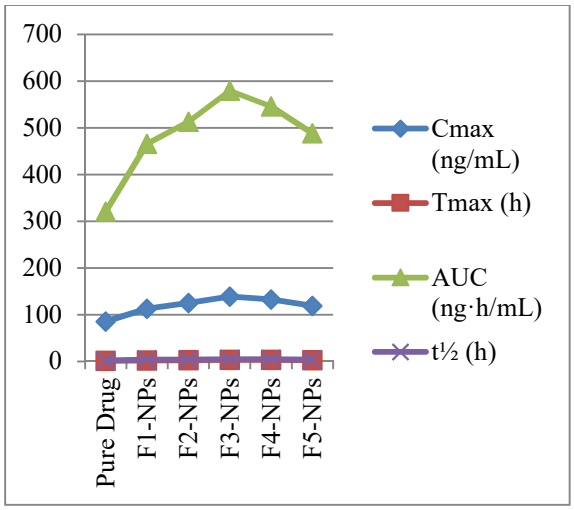

Figure 5: Pharmacokinetic Parameters of Rivastigmine Formulations

4.5. Brain Targeting Efficiency

The brain targeting efficacy of rivastigmine-loaded PLGA nanoparticles was measured by the comparison of drug concentration in the brain and plasma and calculation of brain targeting index (BTI). The formulations of nanoparticles were found to have significant higher drug concentrations in brain tissue than in the pure drug, as evidence of enhanced drug penetration through the BBB. Simultaneously, the plasma concentrations were comparatively lower in nanoparticle treated groups, indicating a decrease in peripheral distribution and an increase in localization in the brain. F3 was the most concentrated in the brain and had the highest BTI value among all the formulations, and this could be due to the fact that it possessed an optimized particle size and surface properties that promoted the absorption into the brain. In comparison, the pure drug was poorly targeted as it accumulated less in the brain with a low BTI. On the whole, these results validate the idea that PLGA nanoparticles have an enormous positive impact on the brain-specific delivery of rivastigmine and increase its targeting capacity.

Table 5: Brain and Plasma Drug Concentration and BTI

Formulation	Brain conc. (µg/g)	Plasma conc. (µg/mL)	BTI
Pure Drug	0.85 ± 0.06	2.40 ± 0.15	0.35 ± 0.03
F1-NPs	1.72 ± 0.10	2.05 ± 0.13	0.84 ± 0.05
F2-NPs	2.05 ±	1.96 ± 0.12	1.05 ±

	0.12		0.06
F3-NPs	2.48 ± 0.14	1.82 ± 0.11	1.36 ± 0.08
F4-NPs	2.30 ± 0.13	1.88 ± 0.12	1.22 ± 0.07
F5-NPs	1.90 ± 0.11	1.99 ± 0.13	0.96 ± 0.06

SEM = standard error of mean; Mean brain concentration = 1.88 µg/g; Mean plasma concentration = 2.02 µg/mL; Mean BTI = 0.96; Values are expressed as Mean ± SEM (n = 6).

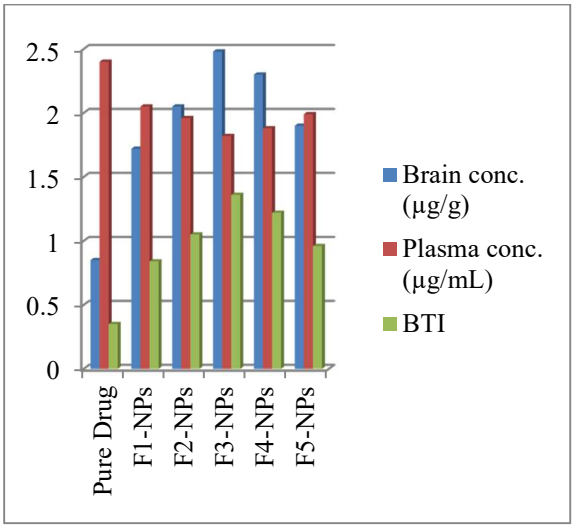

Figure 6: Brain and Plasma Drug Concentration and BTI

4.6. Behavioral and Therapeutic Efficacy in AD Model

The rivastigmine-loaded PLGA nanoparticles were tested on memory and cognitive performance tests of AD model and compared to traditional rivastigmine to establish their therapeutic efficacy. Nanoparticles formulations were found to improve significantly animal learning ability and memory retention in terms of shorter escape latency time and longer time in target quadrant. F3 was the only formulation that was maximally effective in improving behavioral parameters, which demonstrates excellent brain delivery and durable drug effect. On the contrary, when animals were administered with traditional rivastigmine, cognitive improvement was only moderate because it was cleared rapidly and had poor brain penetration. All the tests proved that disease control animals performed poorly, which proved effective induction of cognitive impairment. On the whole, nanoparticle-based rivastigmine showed a better therapeutic effect in comparison to the pure drug because it promoted memory and cognitive outcomes in the AD model.

Table 6: Effect of Rivastigmine Formulations on Memory and Cognitive Performance

Group	Escape latency (s)	Time in target quadrant (s)	Discrimination index
Normal	24.6 ± 1.8	38.4 ± 2.1	0.72 ± 0.04
Disease Ctrl	52.8 ± 2.4	16.2 ± 1.5	0.31 ± 0.03
Pure Drug	36.5 ± 2.0	27.6 ± 1.8	0.54 ± 0.04
F3-NPs	28.4 ± 1.7	34.9 ± 2.0	0.68 ± 0.05

SEM = standard error of mean; Mean escape latency = 35.6 s; Mean time in target quadrant = 29.3 s; Mean discrimination index = 0.56; Values are expressed as Mean ± SEM (n = 6).

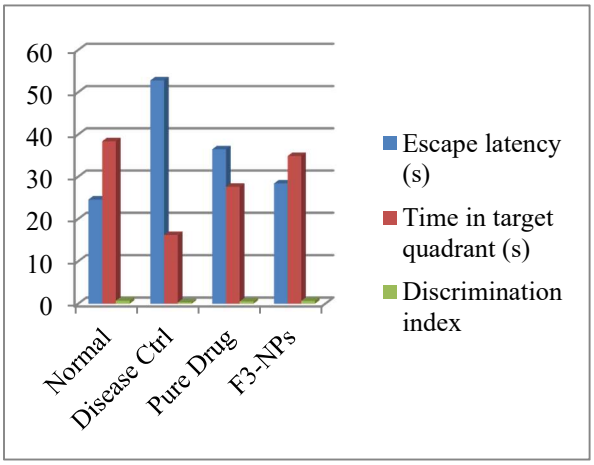

Figure 7: Effect of Rivastigmine Formulations on Memory and Cognitive Performance

5. Discussion

The current paper has shown that rivastigmine-PLGA nanoparticles can be effectively prepared with appropriate physicochemical characteristics to target the brain. The narrow size distribution and low particle size of the nanoscale particle size is evidence of a homogeneous and stable formulation, which is necessary in reproducible biological performance. The zeta potential was moderately negative, which acted as a stabilizing factor of the particles by avoiding aggregation of particles, and thus preserved the integrity of dispersion during circulation. Spherical and smooth-surfaced nanoparticles with a morphological study indicated that the drug could be

easily encapsulated within the polymeric matrix. The cumulative results of these formulations point to the fact that the chosen mode of preparation was suitable in the creation of a stable and effective nanosystem. There was a significant correlation between the physicochemical properties and brain-targeting efficiency. The nanoparticles in the optimal size (around 150 200 nm) are known to cause transportation across the BBB via endocytic and transcytotic pathways. The charge on the surface also contributed to cellular interaction with the endothelial membranes to facilitate uptake without triggering the rapid clearance. Optimized formulation (F3) showed better brain concentration and BTI and this can be explained by the fact that it had a balanced polymer-drug ratio and good surface properties. The results indicate the relevance of optimization of formulation in the pursuit of an effective brain delivery.

This increase in the pharmacokinetic characteristics also contributes to the beneficial difference between nanoparticle-deliberated and traditional rivastigmine therapy. Nanoparticle formulations demonstrated maximum plasma concentration, time to peak concentration, AUC, and t½, which were higher compared to the pure drug. This altered profile is an indicator of sustained drug release of the polymeric matrix and low rate of elimination. Increased systemic exposure and increased circulation time are the key elements in maintaining therapeutic drug levels in the brain. Moreover, biodistribution investigations showed that rivastigmine was accumulated more in brain tissue and lower plasma concentration, which shows that the drug is localized to the target site and limited peripheral exposures. These improvements were proven to have therapeutic relevance in studies of behavioral and cognitive performance in the AD model. The animals receiving the optimized formulation of nanoparticles performed much better in learning and retaining memory as compared to those which were given conventional rivastigmine. The fact that the escape latency is shorter and the time spent in the target quadrant is longer proves successful reversal of cognitive deficits. The latter can be explained by an increase in brain bioavailability and a prolonged cholinesterase inhibition obtained by the help of nanoparticles delivery. Accordingly, the formulation did not only enhance the pharmacokinetic and biodistribution parameters, but also delivered rational functional gains.

The current PLGA nanoparticle system has some unique benefits, when compared to the rivastigmine systems that have been reported previously, e.g. liposomes, solid lipid nanoparticles, and polymeric micelles. PLGA is biodegradable, biocompatible and pharmaceutical use approved and is therefore appropriate in the long term administration. The sustained release characteristics and high brain targeting efficacy of this work is comparable or better than the previous systems in addition to offering better stability and formulation freedom. In spite of all these encouraging results, there are limitations of the study. This was tested in an animal model and direct extrapolation into human therapy is yet to be validated. Toxicity in the long term, immunogenicity, and high-scale stability were not determined and should be studied in the future. Moreover, surface modification using targeting ligands may also be used to increase specificity and transport across the BBB. The future outlook involves the discussion of the ligand-mediated targeting approaches, the studies of chronic treatment, and the studies of clinical translation in order to verify the safety and effectiveness in humans. Altogether, the current paper gives a solid ground to the use of nanoparticle-based rivastigmine delivery as a possible approach to better AD treatment.

Conclusion

The current study was able to achieve the formation of rivastigmine-loaded PLGA nanoparticles as a viable brain-targeted drug delivery system to AD. The optimized nanoparticles formulation had appropriate physicochemical characteristics, such as nanoscale particle size, narrow polydispersity, and stable surface charge, which guarantee the stability of formulation and good drug encapsulation. The ability of the polymeric matrix to release the drugs in a sustained and controlled manner was a pointer of the long-term therapeutic effect and low dosing rate. Pharmacokinetic analyses revealed that there was a significant enhancement of systemic exposure as indicated by increased Cmax, longer Tmax, increased AUC, and longer half-life as compared to the pure drug. The targeting capacity of the nanoparticulate system was further supported by biodistribution outcome which showed high drug concentration in brain tissue and also increased brain targeting index and decreased plasma concentration. Notably, behavioral assessment of the Alzheimer disease model showed that there was an improvement in the memory and cognitive ability in animals receiving the optimized nanoparticle formulation relative to the traditional rivastigmine treatment. These advancements can be explained by the better BBB penetration, constant drug release, and the augmented drug accessibility at the neuronal points of action. In general, this paper demonstrates the possible use of nanoparticles made of PLGA to address significant

shortcomings of the conventional rivastigmine delivery, such as rapid clearance and poor brain bioavailability. Though these results are encouraging, additional studies are needed to determine long-term safety, chronic efficacy and large-scale stability. Also, the strategy of surface modification can be considered in order to achieve even greater specificity of targeting. Finally, the use of rivastigmine- PLGA nanoparticles can be considered as a promising and reasonable solution to enhance the therapeutic results in AD and as a worthy foundation of future evolution of brain-targeted drug delivery system.

Abbreviation

AD	Alzheimer's Disease
BBB	Blood–Brain Barrier
PLGA	Poly (lactic-co-glycolic acid)
PVA	Polyvinyl Alcohol
PBS	Phosphate Buffer Saline
DLS	Dynamic Light Scattering
PDI	Polydispersity Index
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
AUC	Area Under the Curve
Cmax	Maximum Plasma Concentration
Tmax	Time to Reach Maximum Concentration
t½	Half-Life
BTI	Brain Targeting Index
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
IAEC	Institutional Animal Ethics Committee
SEM (stats)	Standard Error of Mean

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