

RESEARCH ARTICLE

Development and Evaluation of Riluzole Loaded PLGA Nanoparticles for Improved Permeability across BBB

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ABSTRACT

Blood-brain barrier is a physiological barrier that prevents drugs from reaching the brain or being bioavailable for treating brain disorders. Degenerative diseases like amyotrophic lateral sclerosis (ALS) seriously impact our daily lives. The Food and Drug Administration (FDA) approved this drug to treat ALS as a chemical derivative of 2-amino-6-[tri-fluoro-methoxy] benzo-thiazole. In order to improve the drug's therapeutic efficacy, poly-(lactic-co-glycolic acid) nanoparticles (PLGA) were loaded with riluzole and deposited using emulsifying solvent deposition. A design expert software program was used to optimize formulation parameters, including polymer concentration, surfactant concentration, and stirring speed, based on the particle size, zeta potential, and entrapment efficiency responses. Three formulations were taken forward for further study based on the results of 20 trials. Compared to differential scanning calorimetry and fourier transform infrared spectroscopy (FTIR) studies performed beforehand, there were no significant interactions between RZL and the excipients. Nanoparticles prepared with scanning electron microscopy had a smooth surface and a spherical shape. The particle size distribution ranged from 184 ± 74 nm to a maximum of 204.5 ± 71 . As a consequence, the particle size distribution is relatively narrow, with lower polymer concentrations, and is ideal for drug delivery. A range of -17.3 to -18.2 mV was found for the zeta potential of the nanoparticles. The encapsulation efficiency ranged from 42.61 ± 3.61 to $60.02 \pm 1.94\%$, forming 1:1 to 1:4 drug: Polymer ratios, respectively. Over a period of 22 to 26 hours, the RLZ was continuously released from the nanoparticles. A loading dose may be built by 24% of the drug being released within 3 hours of administration.

Keywords: Polymeric nanoparticles, Riluzole, Blood-brain barrier, Amyotrophic lateral sclerosis, Neurodegenerative disorder, Entrapment efficiency, *In-vitro* drug release.

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INTRODUCTION

Brain is the major organ that differentiates each human being from other living things in this universe. Any defect occurring in this organ due to physical damage, infections, or aging may seriously affect the quality of the individual's life. Amyotrophic lateral sclerosis (ALS) is one among the neurodegenerative diseases that seriously affect the normal functioning of brain. This condition manifests through various symptoms, including involuntary twitching in areas like the arms, legs, shoulders, or tongue, muscle cramps, stiffness or swelling of muscles (spasticity), weakness in muscles located in the arms, legs, neck, or diaphragm, speech difficulties such as slurring or sounding nasal, challenges in chewing, and difficulties in the swallowing process.

The latest developments occurring in the field of pharmaceuticals and healthcare facilities had consistently

enhanced the quality of the treatment of many diseases and disorders, but unfortunately, a few of the ailments could not be achieved due to physiological reasons. ALS and Alzheimer's disease are such conditions in which the complications are deep-seated in the highly protected organ- brain. The physiological barriers that protect the brain from entry of various substances into the cranial cavity. The presence of the specialized set of modified endothelial cells, pericytes and astrocytes together forms tight intercellular junctions and restrict the entry of the substances in the general circulation. This tight intercellular junction is defined as blood brain barrier (BBB), and the presence of this barrier significantly differentiates the brain's environment from the rest of the body's organs. This barrier also hinders the permeability of the drugs for the treatment of drugs for the brain from the general circulation.¹ The damage or rupture of BBB may improve the permeability of drugs but

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also open up for other materials, which may cause damage to the neurons in the brain. This scenario encourages us to explore the possibilities of enhancing the permeability of drugs used for the treatment of brain disorders without disrupting the BBB. Nanoparticles are one among the intelligent tools for enhancing drug delivery to brain and thereby increasing the bioavailability to the brain.

Riluzole (RZL) is a chemically 2- amino-6 [trifluoromethoxy] benzothiazole derivative used for the treatment of ALS and it has complex actions in the nervous system. Riluzole is commonly administered orally, leading to increased protein binding levels. Metabolized in the liver through cycloxylation and glucuronidation, it boasts a half-life of around 12 hours. In recognition of this drug's status as the only disease-modifying agent that has been approved by the Food and Drug Administration (FDA) (US) for the treatment of ALS, this drug has been sanctioned by the FDA (US) for ALS treatment (31).^{2,3} There is some evidence that the drug slows the progression of ALS in some people, possibly by decreasing levels of brain glutamate, which are many times higher in ALS patients. Furthermore, it inhibits the action in a non-competitive manner.

It is recommended that patients swallow riluzole tablets without crushing them or mixing them with soft foods for easier swallowing. Since crushed tablets have stability issues, they should be taken right away.

Unlike other drugs, it doesn't dissolve in water and cannot be administered through percutaneous endoscopic gastronomy (PEG) feeds because it may block tubing.⁴ Cytary amino acids are released post-synaptically⁵ riluzole also inhibits voltage-gated sodium channels and activates g-proteins.⁶ The side effects of riluzole include dizziness, gastrointestinal conditions and liver function changes. The development of the novel targeted drug delivery system for enhancement of the permeability of riluzole across the BBB, which is expected to improve the therapeutic efficacy of this drug. The nano drug delivery systems formulated with various biodegradable polymers were reported to enhance the permeation of the loaded drug across the BBB and improve their therapeutic action. Therefore the aim of this study to develop a polymeric nanoparticle formulation loaded with riluzole and to evaluate the physico-chemical properties of such formulation.

MATERIALS AND METHODS

Chemicals

Chemicals, reagents, and solvents, including RZL and PLGA, were of analytical grade and purchased from Sigma Aldrich.

Methods

Formulation of RZL-PLGA nanoparticles

As reported by Michele Trotta, the emulsification solvent diffusion technique was used to prepare the RZL-loaded polymeric nanoparticles.⁷ A 10 mL organic phase of PLGA and RZL was prepared in DMSO, and a 100 mL aqueous phase of Poloxamer (0.5 g) was prepared in sterilized water. The

aqueous phase was diluted by 1-mL/minute with the organic phase. DMSO was dissipated by maintaining the colloidal suspension of RZL-PLGA nanoparticles at 300°C for 3 hours at 300 rpm. Approximately 30 minutes of centrifugation at 40°C at 12000 rpm was required to obtain the final nano precipitate. In order to eliminate untrapped RZL from nanoparticle surfaces, the pellet was cleaned twice with deionized water. A 10 mL of demineralized water is added to the nanoparticle and then refrigerated until needed.

Characterization of the Nanoparticles

Optimization

A number of variables, such as polymer concentration, surfactant concentration, stirring speed, were found to be major determinants of the particle size and encapsulation efficiency of polymeric nanoparticles loaded with riluzole. CCRD- RSM was used to examine how these three factors affected polymeric nanoparticles' size and encapsulation efficiency. Table 1 provides a detailed description of the design. In accordance with the results of the initial experiments and the feasibility of preparing the nanoparticles at extreme values, a speculative range was chosen for each element.

Fourier-transform infrared analysis

Utilizing a Fourier-transform infrared (FTIR) analysis system (SPECTRUM RX I, Perkin Elmer, USA) facilitates the evaluation of the drug's and polymer's chemical integrity. The procedure involves blending each riluzole (RZL) sample and nanoparticle with 300 to 400 mg of anhydrous potassium bromide, followed by grinding in a mortar and pestle. The resulting sample blend is subjected to a 2000 kg/cm² hydraulic pressure for two minutes using a Jasco MP2 mini-press. In addition, all samples within the 4000 to 400 cm⁻¹ range are scanned at 2 cm⁻¹ resolution to generate FTIR spectra.

Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC-60, Shimadzu, Japan) was used to examine the drug's physical status and its interaction with the polymer. Individual scans were performed for native RZL (2–4 mg) and RZL-PLGA nanoparticles (2–4 mg) in basic aluminum pans. Heating was carried out at a rate of 10°C/min over a temperature range of 50 to 350°C with continuous circulation of nitrogen gas at 65 mL/min throughout the scanning process.

Scanning electron microscopy analysis

RZL-PLGA nanoparticles were assessed with SEM to assess their surface morphology. A carbon adhesive sample holder was used to apply gold coating to powder samples after lyophilization.¹⁰

Particle size analysis

Particle size measurements of riluzole-PLGA nanoparticles (RZL-PLGA-NP) were conducted using a Malvern Zetasizer 3000 HSA (Malvern Instruments, UK). The polydispersity index (PI), representing the width of the size distribution in relation to the mean size, was determined. Measurements were

Table 1: Optimization studies carried out for RLZ-PLGANP with 3 factors against the two responses

Standard	Run	Factor-I	Factor-II	Factor-III	Response-I	Response-II
		A: Polymer concentration	B: Surfactant concentration	C: Speed of rotation	Particle Size (nm)	EE (%)
11	1	2.5	-1.57039	1750	756.4	70.1
3	2	1	5	500	120.23	27.32
7	3	1	5	3000	80.8	46.61
2	4	4	0.1	500	296	52.58
17	5	2.5	2.55	1750	150.22	58.41
10	6	5.02269	2.55	1750	310.54	72.11
12	7	2.5	6.67039	1750	148.36	46.01
9	8	-0.0226892	2.55	1750	0	0
16	9	2.5	2.55	1750	157.81	41.77
14	10	2.5	2.55	3852.24	132.09	48.12
19	11	2.5	2.55	1750	151.62	40
4	12	4	5	500	287.05	56.14
1	13	1	0.1	500	145.33	34.76
8	14	4	5	3000	276.21	71.1
15	15	2.5	2.55	1750	111.36	56.14
20	16	2.5	2.55	1750	156.83	54.09
6	17	4	0.1	3000	300.41	70.55
5	18	1	0.1	3000	138.01	54.6
18	19	2.5	2.55	1750	150.22	51.9
13	20	2.5	2.55	-352.241	387.35	47.43

performed at 25°C using a 10 mm diameter cell. Prior to the measurements, all samples were diluted in double-distilled water to ensure optimal scattering intensity.⁹

Zeta potential

The Malvern Zetasizer 3000 HSA (Malvern Instruments, UK) was used to assess colloidal systems' physical stability and zeta potential (an indicator of electrical charge). A 0.9% w/v sodium chloride solution was employed to adjust the conductivity of RZL-PLGA-NP to 50 IS/cm. pH was kept within the range of 5.5 to 7.5, and 20 V/cm was applied as the applied field strength.⁹

Drug-encapsulation efficacy determination

RZL-PLGA-NP were encapsulated in 0.1 M hydrochloric acid suspension to assess their drug encapsulation efficiency. Both the supernatant (S1) and solid deposits were collected after centrifugation (MIKR022, HEETTICH, Germany) for 50 minutes. After re-dispersing the solid deposit in a 0.3% sodium dodecyl sulfate (SDS) solution for 10 mL, centrifugation was performed to collect the supernatant (S2).⁹ After that, formulas were used to determine the percentage of drug encapsulation efficiency of the nanoparticles (1):

Where,

- EE = Entrapment Efficiency;
- WT = total amount of charged drug;
- WS_1 = Supernatant drug concentration after first centrifugation;

- WS_2 = Supernatant drug concentration after second centrifugation.

In-vitro drug release study

A phosphate-buffered saline (PBS) medium with a pH of 7.4 was used to evaluate nanoparticle formulations. A sample of nanoparticles (0.5 mL) was placed inside a dialysis bag, followed by a solution of PBS in the amount of 50 mL. A gentle shake of 100 rpm at 37°C was performed for 72 hours. Throughout the experiment, fresh PBS was added at predetermined intervals in aliquots of 2 mL. Additional fresh PBS solutions were added to the external buffer solutions at 8 hours, 24, and 48 hours after initiation. Spectrophotometric analysis at 275 nm was performed to determine the concentration of RZL in the dispersion medium. To simulate *in-vivo* conditions, we also introduced RZL-PLGA-NP and human plasma (1:1 v/v) into a dialysis bag.

RESULTS AND DISCUSSION

Preparation of RLZ-PLGA-NPs was done by solvent diffusion emulsification. Encapsulation efficiency was enhanced through the use of PLGA polymer and stabilization of the drug. The emulsion was stabilized with poloxamer as surfactant. To assist in quenching the dispersed organic solvent right into the aqueous phase as well as to saturate the organic solvent in water, an excess quantity of water was used when preparing the emulsion for solvent diffusion. To judge reproducibility and uniformity, the preparation was done in triplicate.

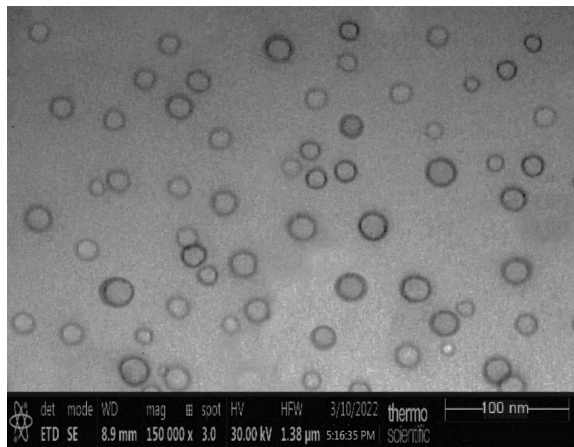


Figure 1: Scanning electron microscopic photographs of RLZ-PLGA-NPs

FTIR and DSC tested the drug and the polymers for identity and purity. The mixtures were also studied for incompatibilities, if any. The studies indicated that there were no sign of incompatibilities. A further minor shift in the melting point (114.2°C in the mixture) of riluzole from 117.1°C (in its pure form) may be due to a change in the crystalline structure, which cannot interfere in the integrity of the formulation. Hence the process of the development of trial formulations were conducted.

The nanoparticles were prepared by emulsification solvent diffusion technique and the nanosuspensions obtained were subjected to various physiochemical evaluations and the results obtained are given below.

The morphology of the nanoparticles was studied under SEM and found to be spherical in shape and the surface was found to be much smoother. The morphological structure of Riluzole loaded PLGA nanoparticles is shown in Figure 1. This suggests the quick escape of organic solvents deposited on the surface during the emulsification process. However, the strength of the particles are found to be isolated and unbroken. The particle sizes measured by this method were around 200 nm. Further, there were no signs for the presence of the free drug crystals. This may be due to the washing and redispersion of the nanoparticles, the unloaded drug molecules might have been removed from the preparation.

Estimating the size distribution study of the prepared nanoparticles suggested an average particle size of 184 ± 74 nm to a maximum of 204.5 ± 71 . This further suggests that the particle size distribution is relatively narrow with lower concentrations of the polymers and ideal for the delivery for drugs into the brain.

The zeta potential of the RLZ-PLGA-NPs were found to be within a range of -17.3 to -18.2 mV. This indicates that the prepared RLZ-PLGA-NPs were found to be negative and optimally high, which may be capable of generating higher intraarticular repulsive forces sufficient enough to maintain the stability and prevent the aggregation of the particles on storage. The SEM studies also corroborate with the exhibition of no aggregates or agglomeration.

Table 2: Physico-chemical evaluation of riluzole loaded PLGA nanoparticles

Code for formulation	Size of the particles (nm)	Zeta potential (mV)	Entrapment efficiency (%)
RLZPLGANPO-01	182.2 ± 74	-18.2	42.61 ± 3.61
RLZPLGANPO-02	196.2 ± 11	-18.7	55.62 ± 1.25
RLZPLGANPO-03	203.1 ± 21	-17.9	59.21 ± 2.44
RLZPLGANPO-04	204.5 ± 71	-17.3	60.02 ± 1.94

The encapsulation efficiency of the RLZ-PLGA-NPs ranged from 42.61 ± 3.61 to $60.02 \pm 1.94\%$, forming 1:1 to 1:4 drug: Polymer ratios, respectively. Although the drug concentration was kept constant, a considerable increase in the encapsulation efficiency was noted with the increase in the polymer. However, the parallel increase in the particle size with an increase in the polymer concentration had practically limited in enjoying the benefit of enhancement of the encapsulation efficiency by altering this parameter. The details of Physico-chemical evaluation of various batches of Riluzole loaded PLGA nanoparticles is given in Table 2.

The *in-vitro* release studies of the RLZ from RLZ-PLGA-NPs were determined and found to release the drug continuously from 22 to 26 hours. About 24% of the drug was released within the first 3 hours in all the tested formulations, which may help build the loading dose. However, variations in the pattern was recorded in various formulations depending upon the differences in the formulation parameters. The analysis of the data obtained from the *in-vitro* release of the drug suggests that the RLZ-PLGA-NPs follow zero-order kinetics. A similar study carried out on the release of RLZ from nanostructured lipid carriers reported the release of around 75% of RLZ in 24 hours.¹²

CONCLUSION

The RLZ-PLGA-NPs had successfully demonstrated the properties that are idealistic for delivery of not only RLZ but any such similar drugs having associated properties. The data obtained from this research suggest the dosage form's reliable stability and applicability. It is also further appropriate for a wide range of modes of administration from oral to IV injection, as the particle size range supports for the same. However, RZL is not administered parenterally.

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