DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIERS FOR OPTIMIZED DRUG DELIVERY OF TEMOZOLOMIDE IN GLIOBLASTOMA MULTIFORME

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ABSTRACT

The management of glioblastoma multiforme (GBM), a cancer derived from grade IV astrocytoma, presents significant challenges due to its chemoresistance and the complexities associated with drug delivery across the blood-brain barrier (BBB). Nanostructured systems, notably nanostructured lipid carriers (NLCs), offer a promising solution to these challenges by enhancing drug delivery and bioavailability, while reducing systemic toxicity and adverse effects. This study examines the development of a cationic NLC encapsulating temozolomide (TMZ), the preferred drug for GBM treatment, aiming to enhance the drug's therapeutic efficacy and cerebral bioavailability. It also explores the impact of temperature on nanoparticle formation. The optimal formulation demonstrated stability and desirable characteristics over the study period, suggesting its potential for effective TMZ administration. A temperature-dependent analysis, utilizing a gradient from 25°C to 70°C in 5°C increments, revealed notable changes in particle size, distribution, and zeta potential, underscoring the formulation's thermal sensitivity. A notable increase in average particle size from 145 nm at 25°C to 197 nm at 70°C was observed, indicating potential structural or molecular interaction alterations within the NLC system in response to temperature elevation.

Keywords: Lipid nanoparticles. Nanostructured lipid carriers. Drug delivery systems. Glioblastoma Multiforme.

1 INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults, classified as grade IV astrocytoma. Approximately 90% of cases manifest as primary tumors in individuals aged 62 and older, while the remaining 10% derive from secondary gliomas[1]. Diagnosis relies on laboratory tests combined with imaging techniques, such as computed tomography or magnetic resonance imaging [2.] The current therapeutic protocol for GBM involves surgical resection followed by administration of the first-choice drug, temozolomide (TMZ), in conjunction with radiotherapy. However, GBM is considered an incurable disease due to the development of resistance to both chemotherapy and radiotherapy.

These factors contribute to the low survival rates of patients with this neoplasm. Despite recent therapeutic advancements, GBM treatment remains a significant challenge, especially considering the existence of the blood-brain barrier (BBB), that hampers the delivery of chemotherapeutic agents to brain tissue, which contributes often for increased drug dosages for treatment, leading to various complications that compromise both the patient's overall health condition and treatment efficacy[1.]

To address the inherent challenges of current treatments for glioblastoma multiforme (GBM), research into alternative technologies ensuring more effective drug delivery to brain tissue has been encouraged. Within this context, nanotechnology emerges as a promising solution, particularly through the use of nanocarriers for drug delivery. The nanostructured lipid carriers (NLCs) in special, are noteworthy for their capability to protect drugs from pharmacokinetic processes such as degradation, renal elimination, and metabolism. Furthermore, they enhance cerebral biodistribution of the carried drug due to their nano-scale size, which facilitates easier crossing of the BBB. This capability not only reduces the amount of drug present in healthy tissues but also diminishes adverse effects, contributing to a more targeted and efficient treatment strategy[3.]

2 MATERIAL & METHODS

The emulsions of nanostructured lipid carriers (NLC) were prepared by the fusion-emulsification method[4]. The best configuration of the formulation between all the dispersions prepared was a mixture of oleic acid (0.07 g), Compritol® ATO 888 (0.03 g), and the surfactant cetyltrimethylammonium bromide (CTAB) (0.05 g). The lipid phase composed of temozolomide 0.1% w/w), oleic acid (AO) and Compritol® ATO 888 were combined and heated until 80 ºC. Then the heated aqueous phase (ultra-purified water) (80 ºC) was added to the lipid phase. This mixture was stirred for 30 s, using a magnetic stirrer, resulting in a pre-emulsion. The final formulation was obtained using an ultrasonic processor (Q700 Sonicator, Qsonica, Newtown, CT, USA), at intervals of 60 s, for 10 min (amplitude, 90%; energy source, 700 W; probe diameter, 1/16).

The hydrodynamic diameter (HD) and the polydispersity index (PdI) were measured by dynamic light scattering (DLS). The zeta potential (ZP) was assessed by the electrophoretic mobility method using Zetasizer equipment (Nano ZS, Malvern, UK). The formulations were diluted in deionized water (1:10 v/v) and the analyses were made in triplicates.

3 RESULTS & DISCUSSION

Initially, it was observed that the NLC loaded with TMZ exhibited a particle size of 171.5 nm, an PdI of 0.27 and a zeta potential of +58 mV, indicating satisfactory stability of the physicochemical properties. These parameters remained relatively constant throughout the study period as shown in Table 1.

Table 1. The hydrodynamic diameter (HD), polydispersity index (PdI) and zeta potential (ZP)

NLC-TMZ	Hydrodynamic diameter (nm)	Polydispersivity index (-)	Zeta Potencial (mV)
Day 1	171,5 ± 1,04	0,27 ± 0,001	58,8 ± 1,04
Day 7	173,9 ± 0,92	0,31 ± 0,008	58,5 ± 2,44

Analysis of the size distribution pattern of the formulations revealed a monomodal profile (Figure 1), as evidenced by the low IPd. This finding suggests satisfactory uniformity in the distribution of particles in the formulations tested. The consistency of this profile over time reinforces the stability of the formulations and the uniformity of the particle properties[4].

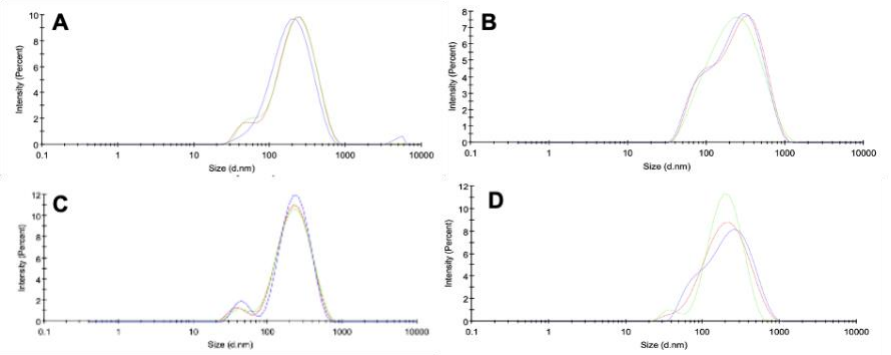

Figure 1. Particle size distribution after 24 hours (A and C) and 7 days (B and D).

Also, this study evaluated the influence of temperature on particle size parameters, polydispersity index (PdI), and zeta potential using a temperature gradient approach from 25°C to 70°C in 5°C increments for all the dispersions that were first developed.The findings demonstrated significant changes in particle size, size distribution, and zeta potential in response to temperature variations, highlighting the formulation's thermal sensitivity. A consistent increase in average particle size was observed with rising temperature, from 145 nm to 197 nm between 25°C and 70°C, suggesting potential changes in formulation structure or molecular interactions within the lipid nanoparticle system due to temperature elevation. This trend may be attributed to the expansion or reorganization of lipid structures at higher temperatures when there's variation in the concentration of the formulation compounds.

Conversely, a notable decrease in PdI was recorded with increasing temperature, indicating a more uniform and homogenous particle size distribution at higher temperatures. The reduction in PdI suggests decreased particle size heterogeneity and a more uniform distribution, pointing to enhanced stability and uniformity in the formulation at elevated temperatures, potentially due to increased interaction among formulation components and structural rearrangements at higher temperatures.

Moreover, the evaluation of zeta potential indicated relatively minor variations compared to PdI and particle size in response to temperature changes. Zeta potential values decreased from 62.4 mV to 46.3 mV between 25°C and 70°C, implying possible alterations in the surface charge of particles due to temperature shifts. This decrease in zeta potential may signify a reduction in electrostatic stability of the particles at higher temperatures, which could impact interactions with the surrounding environment and overall formulation stability, as demonstrated in the Figure 2.

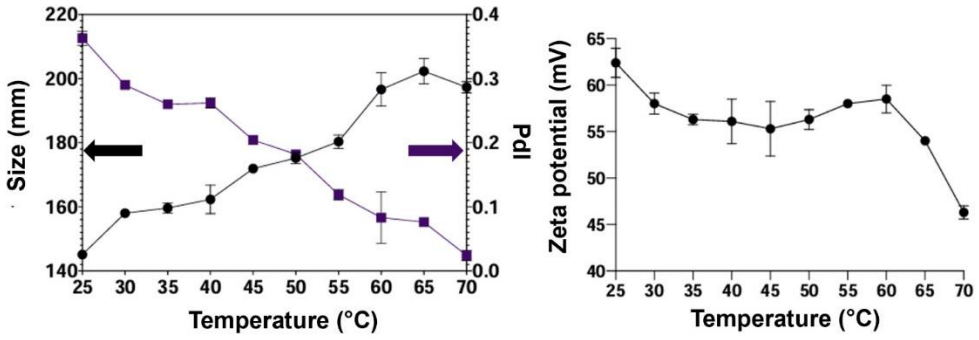

Figure 2. Influence of temperature on particle size, polydispersity index (PdI) and zeta potential of NLC-TMZ..

4 CONCLUSION

The development of NLC formulations for the delivery of TMZ is a promising approach to the optimization of targeted therapy for the treatment of GBM. The use of specific components, such as oleic acid, compritol and CTAB, demonstrated the stability of the physicochemical attributes of the NLCs given over time, including particle size, size distribution and zeta potential, highlighting the robustness and potential viability of these formulations. The observations of more heterogeneous particle size distributions at higher temperatures indicate the sensitivity of the formulations to temperature variations, providing a valuable perspective for refining formulation strategies and optimizing storage conditions.

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