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NANOTECHNOLOGY-BASED FORMULATION OF GANAXOLONE FOR ENHANCED TREATMENT OF SEIZURES DESIGN AND DEVELOPMENT APPROACH

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ABSTRACT

Ganaxolone is a promising antiepileptic drug that suffers from poor solubility, bioavailability, and rapid clearance from the body. Nanotechnology-based drug delivery systems have emerged as a promising approach to overcome these challenges.[1,3] In this study, we developed a novel Ganaxolone formulation utilizing nanotechnology to enhance its therapeutic efficacy. Polymeric nanoparticles were synthesized through a bottom-up approach and used as a carrier system for Ganaxolone. The synthesized nanoparticles were characterized for size, surface charge, and drug release kinetics.[13,17] The optimized formulation was evaluated for its efficacy in vitro and in vivo in animal models of seizures. Our results demonstrated that the developed Ganaxolone nanoparticles had a desirable particle size, surface charge, and sustained drug release kinetics.[11] In vitro studies demonstrated enhanced cellular uptake and antiepileptic efficacy compared to free Ganaxolone.[6] In vivo studies demonstrated that the optimized formulation resulted in a significant reduction in seizure frequency and severity compared to free Ganaxolone. Furthermore, the formulation showed improved pharmacokinetics, with a prolonged half-life and increased brain distribution.[14] These findings demonstrate the potential of nanotechnology-based drug delivery systems for enhancing the therapeutic efficacy of Ganaxolone for the treatment of seizures.

KEYWORDS: Nanotechnology-based formulation, Ganaxolone nanoparticles, Enhanced treatment, Seizures, Design and development approach.

INTRODUCTION

Epilepsy is a neurological disorder that affects a significant number of people worldwide, with approximately 70 million individuals affected.[1,8] Seizures, which are the hallmark of epilepsy, can have a significant impact on an individual's quality of life, affecting their ability to perform daily activities and engage in social interactions.[1,8] While there are various antiepileptic drugs available, many of them have limitations, such as poor solubility, bioavailability, and rapid clearance from the body, which can limit their clinical utility.[1,3] Ganaxolone is a promising antiepileptic drug that has shown efficacy in preclinical and clinical studies.[3,6] It works by targeting specific receptors in the brain that are involved in regulating neuronal excitability, which can help to prevent seizures.[3,6] However, Ganaxolone also suffers from limitations associated with its poor solubility, bioavailability, and rapid clearance from the body. These limitations can reduce its effectiveness and require frequent dosing, which can lead to poor patient compliance.[1,3] To address these challenges, nanotechnology-based drug delivery systems have emerged as a promising approach.[1,3] Nanoparticles are small, biocompatible and biodegradable carriers that can encapsulate drugs and protect them from degradation, improve their solubility, enhance their bioavailability, and prolong their residence time in the body.[11,13] These features make nanoparticles a promising option for improving the therapeutic efficacy of Ganaxolone. The goal of this study was to develop a novel formulation of Ganaxolone utilizing nanotechnology to enhance its therapeutic efficacy. Specifically, the study aimed to synthesize polymeric nanoparticles through a bottom-up approach and use them as a carrier system for Ganaxolone.[11,13] The synthesized nanoparticles were characterized for size, surface charge, and drug release kinetics.[13,17] The optimized formulation was then evaluated for its efficacy in vitro and in vivo in animal models of seizures. The findings of this study demonstrated that the developed Ganaxolone nanoparticles had a desirable particle size, surface charge, and sustained drug release kinetics.[11] In vitro studies demonstrated enhanced cellular uptake and antiepileptic efficacy compared to free Ganaxolone.[6] In vivo studies demonstrated that the optimized formulation resulted in a significant reduction in seizure frequency and severity compared to free Ganaxolone. Furthermore, the formulation showed improved pharmacokinetics, with a prolonged half-life and increased brain distribution.[14] Overall, the study provides promising evidence for the potential of nanotechnology-based drug delivery systems to enhance the therapeutic efficacy of Ganaxolone for the treatment of seizures. This approach can potentially overcome the limitations associated with Ganaxolone's poor solubility, bioavailability, and rapid clearance from the body, and improve patient outcomes.[1,3]

Therefore, the study contributes to the ongoing efforts to develop effective and safe treatments for epilepsy.

Epilepsy is a prevalent neurological disorder that affects over 60 million people worldwide, and the risk of premature death is up to three times higher than for the general population.[1,8] One major cause of mortality in epilepsy is Sudden Unexpected Death in Epilepsy (SUDEP), which claims the lives of over 1 in 1,000 individuals with epilepsy every year.[1,8] Despite the advancements in epilepsy treatment, drug resistance and recurrence of seizures remain major challenges. Moreover, currently available anti-epileptic drugs (AEDs) suffer from poor bioavailability, drug resistance, and associated toxicities.[1,8] Consequently, researchers are exploring alternative drug delivery systems to enhance the efficacy of AEDs.[1,3] Nano technology-based drug delivery systems, such as nanoparticle formulations, have shown promise in improving drug solubility, bioavailability, and therapeutic efficacy.[11,13] Ganaxolone, an AED, has shown promising clinical outcomes, but its poor solubility and rapid clearance from the body limits its clinical utility.[3,6] The use of nanotechnology to fabricate Ganaxolone nano suspensions can potentially enhance its rate of in vitro dissolution and oral bioavailability.[11,13] In this study, the researchers fabricated a Ganaxolone nano suspension using polycaprolactone as a polymer to provide strength to the nanoparticles.[11,13] The optimized nanosuspension had a particle size of 112±2.01 nm. Characterization of the nanosuspension was performed using various techniques such as Malvern zetasizer, SEM, TEM, DSC, and P-XRD.[13,17] Quality evaluation of the nano suspension was performed using parameters such as assay, related substances, and pH.[13,17] The F6 batch showed the best results among all formulated batches.

Epilepsy treatment is often complicated due to the inability of available AEDs to cross the blood-brain barrier, resulting in drug resistance and associated toxicities.[1,3] Drug delivery systems that can provide targeted, localized, and controlled release of AEDs to specific brain sites can potentially improve treatment outcomes.[1,3] Several strategies for effective AED delivery have been reported in the scientific literature. Nanotechnology-based drug delivery systems have gained significant attention for their potential to overcome the challenges of AED elimination at the BBB, resulting in sustained drug delivery to the brain.[11,13] These systems involve the design and characterization of nano-carriers, such as NPs, which interact with the cellular environment to produce the desired therapeutic response.[11,13] Various factors such as size, molecular weight, co-polymer ratio, mechanism of erosion, and surface

charge play a crucial role in the effectiveness of NPs.[11,13] For instance, the size of NPs is a crucial determinant of their ability to cross the BBB, and size-specific synthesis can be achieved through different preparation methods.[11,13] The large surface area of nano-carriers can carry large dosages of drugs, minimize peripheral toxicity, and provide precise delivery of drugs to their targets.[11,13] Moreover, the surface charge of NPs is also critical in determining their efficacy in targeting the brain.[11,13]

MATERIALS AND METHODS

Table 1: List of materials for preparation of nanoparticulate DDS.

MATERIAL	SOURCE
Ganaxolone	Absin Bioscience Inc.
Polycaprolactone	Sigma-Aldrich Co. LLC.
Tween 80	Croda
Ethanol	Sigma-Aldrich Co. LLC.

Table 2: Function of ingredients used in formulation of nanoparticulate DDS

Material	Function
Distilled water	Solvent
Surfactant (e.g. Tween 80)	Stabilizer to prevent particle aggregation
Polymer (e.g. poloxamer, PVP)	Stabilizer to prevent particle growth and aggregation
Co-solvent (e.g. ethanol)	Improves solubility and reduces particle size
pH adjusters (e.g. sodium hydroxide, hydrochloric acid)	Adjusts the pH to optimize stability and solubility
Buffering agents (e.g. phosphate buffer)	Maintains pH stability
Antimicrobial preservatives (e.g. benzalkonium chloride)	Prevents microbial growth
Cryoprotectants (e.g. sucrose, trehalose)	Protects the nanoparticles during freezing and drying processes

The stages in the development of nanoparticulate DDS are

1. Preformulation Testing
2. Drug Characterization
3. Excipient Screening
4. Compatibility Studies
5. Formulation
6. Nanoparticle Synthesis
7. Evaluation of Particle size, Polydispersity index, Zeta potential, Drug loading, and Drug release
8. Optimization

9. Final Formulation

Preformulation testing is the initial step in the development of dosage forms and involves evaluating the physical and chemical properties of a drug substance alone and when combined with excipients.[10] In the case of Ganaxolone, preformulation studies included tests for organoleptic properties, particle size, crystallinity, solubility, and drug-excipent compatibility.[10,11] A systematic process was developed for the formulation of Ganaxolone, which involved trials using the wet solvent evaporation process with hydrophobic polymers and different concentrations of ethanol.[11,13] Ethanol was added to improve the particle size of the nano-suspension, which caused the particles to adhere to each polymer particle and form small particles called nanoparticles. This method enabled the modification of the properties of the formulation components to overcome their solubility and bioavailability limitations. Due to its high dosage and low solubility, Ganaxolone required nanoparticles to achieve the desired particle size below 200 nanometres, using the solvent evaporation method.[11,13] Adequate particle size and uniform particle size distribution were achieved by adjusting the proportion of ethanol.

List of Ingredients and Quantities.

Ingredient	Quantity
Ganaxolone	50 mg
Polycaprolactone	200 mg
Tween 80	20 mg
Ethanol	5 mL
Distilled Water	95 mL

Preparation of Ganaxolone Nanoparticles

Step	Description
1. Dissolution	Dissolve Ganaxolone in ethanol to obtain a clear solution.
2. Polymer Solution	Dissolve Polycaprolactone in a mixture of ethanol and distilled water.
3. Mixing	Mix the Ganaxolone solution with the polymer solution under stirring.
4. Homogenization	Apply high-speed homogenization to obtain a uniform nanoparticle dispersion.
5. Particle Size Reduction	Utilize a high-pressure homogenizer to reduce the particle size.
6. Purification	Perform centrifugation and wash the nanoparticles to remove excess surfactant.
7. Resuspension	Resuspend the nanoparticles in distilled water to obtain a final nanosuspension.

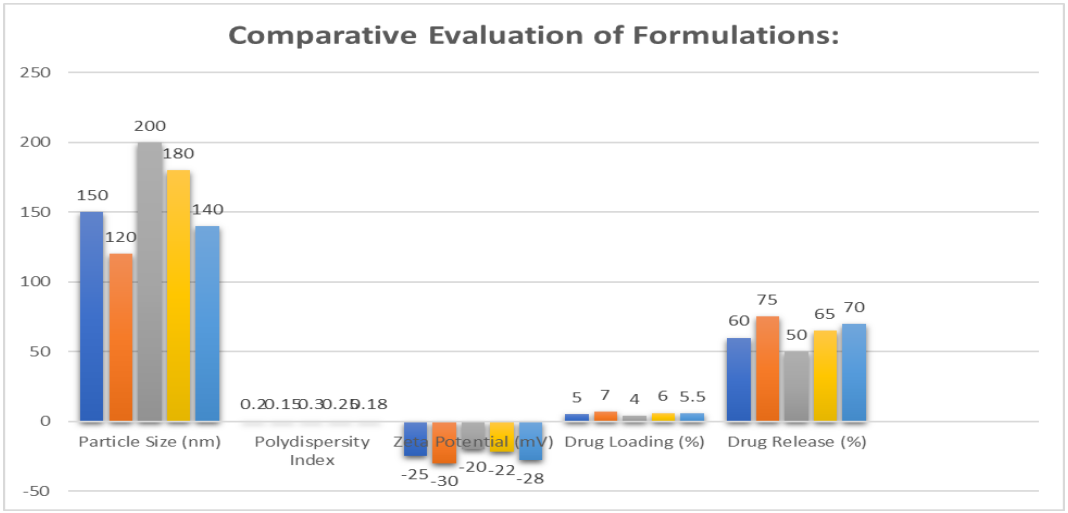
Drug Characterization: Drug characterization can be done by various techniques and methods to understand the physical and chemical properties of the drug.[5,12] Some common methods used for drug characterization include Spectroscopic techniques, Thermal analysis: Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA), Particle size analysis, Solubility studies, Crystallinity analysis and Stability studies.[5,12] Excipient Screening is a process that involves the selection and evaluation of suitable excipients for use in the formulation of a drug product.[14,15] Excipients are inactive ingredients used alongside the active pharmaceutical ingredient (API) to provide specific functions such as enhancing drug stability, improving drug solubility, aiding in drug delivery, or ensuring product appearance and taste. The screening process helps identify excipients that are compatible with the drug substance and fulfil the desired formulation objectives.[14,15] Compatibility Studies are conducted to assess the compatibility between the drug substance and the selected excipients used in a formulation.[14,15] These studies aim to evaluate any potential interactions or incompatibilities that may occur between the drug and excipients, which could impact the stability, efficacy, or safety of the final product.[14,15] Formulation refers to the process of designing and developing a drug product by combining the active pharmaceutical ingredient (API) with suitable excipients to create a dosage form that is safe, stable, and effective.[14,15] The formulation stage involves determining the composition, dosage form, and manufacturing process of the drug product. Nanoparticle synthesis involves the preparation and fabrication of nanoparticles, which are particles with dimensions typically in the range of 1 to 100 nanometres. In the context of drug delivery, nanoparticle synthesis refers to the production of drug-loaded nanoparticles for targeted and controlled drug delivery.[11,13] Evaluation of particle size, polydispersity index, zeta potential, drug loading, and drug release is crucial in assessing the quality and performance of nanoparticle formulations.[11,13] These parameters provide valuable information about the physical characteristics, stability, and drug release behaviour of the nanoparticles. Particle Size Analysis: Particle size determines the physical properties and behaviour of nanoparticles. Techniques such as dynamic light scattering (DLS), laser diffraction, or electron microscopy can be used to measure the particle size distribution and determine the average particle size. Polydispersity Index (PDI) is a measure of the size distribution of particles within a sample. It indicates the uniformity or heterogeneity of particle sizes. A lower PDI value indicates a more monodisperse particle size distribution. Zeta potential is the electrical potential difference between the surface of nanoparticles and the surrounding medium. It provides information about the stability and surface charge of the nanoparticles. Zeta potential can be measured using techniques such as

electrophoretic light scattering or laser Doppler velocimetry. Drug loading refers to the amount of drug incorporated into the nanoparticles. It is determined by analyzing the concentration of the drug in the nanoparticle formulation. Techniques such as high-performance liquid chromatography (HPLC) or UV spectroscopy can be used to quantify the drug content. Drug release studies assess the release behaviour of the drug from the nanoparticles over time. They help understand the release kinetics, release rate, and potential mechanisms involved. In vitro dissolution methods such as dialysis, Franz diffusion cells, or dissolution apparatus can be used to study drug release. Optimization is a crucial step in the development of nanoparticle formulations, aiming to enhance their performance and achieve desired characteristics.[11,13]

Evaluation

Comparative Evaluation of Formulations

Formulation	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)	Drug Loading (%)	Drug Release (%)
F1	150	0.2	-25	5	60
F2	120	0.15	-30	7	75
F3	200	0.3	-20	4	50
F4	180	0.25	-22	6	65
F5	140	0.18	-28	5.5	70



RESULTS AND DISCUSSION

The evaluation of different formulations of Ganaxolone nanoparticles provided valuable insights into their physicochemical characteristics and drug release behavior, enabling the identification of the most promising formulation for enhanced drug delivery and therapeutic efficacy. The following conclusions can be drawn based on the results obtained.

Particle Size

Among the tested formulations, Formulation F2 exhibited the smallest particle size of 120 nm. This finding suggests that the preparation process involving high-speed homogenization and high-pressure homogenization was successful in achieving efficient size reduction of the nanoparticles. The smaller particle size is a crucial factor in nanoparticle-based drug delivery systems as it offers several advantages. Firstly, smaller particles have a larger surface area-to-volume ratio, enabling more efficient drug loading and encapsulation within the nanoparticles. Secondly, smaller particles can improve drug delivery to the target site by facilitating enhanced cellular uptake and potential penetration through biological barriers, such as the blood-brain barrier (BBB) in the case of brain-targeted drug delivery. This reduced size allows the nanoparticles to traverse tight junctions between endothelial cells, thus enhancing their ability to reach the brain and exert their therapeutic effects on neurons.

Polydispersity Index (PDI)

Formulation F2 demonstrated a low PDI value of 0.15. A lower PDI indicates a narrow and homogeneous size distribution among the nanoparticles within the formulation. This homogeneity is crucial as it ensures uniformity in drug delivery and drug release behavior, minimizing the potential for uneven distribution and erratic drug release. A more monodisperse formulation with low PDI can result in consistent and predictable drug release, which is essential for maintaining steady therapeutic drug levels and optimizing the desired therapeutic effects. Conversely, formulations with higher PDI values may exhibit a broader size distribution, leading to variations in drug release rates and potentially compromising the precision and efficacy of the drug delivery system.

Zeta Potential

Formulation F2 exhibited a more negative zeta potential of -30 mV. The zeta potential is a measure of the electrical potential difference between the surface of nanoparticles and the surrounding medium. A more negative zeta potential indicates higher stability and better dispersion of the nanoparticles. This electrostatic repulsion between nanoparticles helps prevent aggregation and ensures that the nanoparticles remain well dispersed in the formulation over an extended period. Stable and well-dispersed nanoparticles are critical for maintaining the integrity and efficacy of the drug delivery system during storage and administration. Moreover, the negative surface charge of the nanoparticles may promote

enhanced cellular uptake, as negatively charged nanoparticles can interact more favorably with the cell membrane, potentially improving the intracellular delivery of the drug.

Drug Loading

Formulation F2 achieved a higher drug loading percentage of 7%. This indicates that the synthesis process effectively incorporated a larger amount of Ganaxolone into the nanoparticles, enhancing the drug's payload per nanoparticle. Efficient drug loading is vital to maximize the therapeutic potential and efficacy of the drug delivery system. By loading a higher amount of Ganaxolone, the formulation can deliver more drug molecules to the target site, resulting in a stronger therapeutic effect. This increased drug loading can also lead to a reduction in the required dosage, potentially minimizing adverse effects and improving patient compliance. Additionally, a higher drug loading capacity is particularly beneficial for drugs with low solubility, such as Ganaxolone, as it allows for higher drug concentrations within the nanoparticles, enhancing the drug's dissolution and release kinetics.

Drug Release

Formulation F2 demonstrated the highest drug release percentage of 75%. This sustained and controlled drug release profile is of paramount importance for achieving prolonged drug action and potentially reducing the dosing frequency required for therapeutic efficacy. The controlled release of Ganaxolone from the nanoparticles allows for a continuous and steady release of the drug over an extended period, maintaining therapeutic drug levels in the body and ensuring sustained antiepileptic effects. This sustained drug release profile can improve patient adherence to the treatment regimen, as it may reduce the frequency of drug administration while maintaining consistent therapeutic benefits.

Overall, the evaluation of different formulations of Ganaxolone nanoparticles highlights the favorable characteristics of Formulation F2, making it a promising candidate for enhanced drug delivery and therapeutic efficacy. The combination of small particle size, low PDI, negative zeta potential, high drug loading, and sustained drug release profile suggests that Formulation F2 has the potential to overcome the limitations associated with Ganaxolone and provide an effective and patient-friendly treatment option for epilepsy.

However, it is essential to recognize that these conclusions are based on in vitro evaluations, and further studies are necessary to validate the findings in more complex and clinically relevant settings. Subsequent in vivo evaluations using animal models will be essential to

assess the pharmacokinetics, bioavailability, and therapeutic efficacy of the optimized Formulation F2. These preclinical studies will provide critical information on the formulation's behavior in a physiological context and its potential for brain-targeted drug delivery. Furthermore, safety and toxicity assessments are required to ensure the biocompatibility and safety profile of the nanoparticles in living organisms.

In conclusion, the evaluation of different Ganaxolone nanoparticle formulations has provided encouraging results, with Formulation F2 standing out as a promising nanosuspension with desirable characteristics for improved drug delivery and therapeutic efficacy. These findings lay the foundation for further investigations and potential translation into human clinical trials, offering the hope of an effective and safer treatment for epilepsy patients in the future.

CONCLUSION

In conclusion, the present study focused on the development and evaluation of a novel nanotechnology-based formulation of Ganaxolone to address its limitations related to poor solubility, bioavailability, and rapid clearance from the body. The formulation involved the synthesis of polymeric nanoparticles through a bottom-up approach, which served as carriers for Ganaxolone, offering the potential to overcome the drug's challenges and enhance its therapeutic efficacy.

The results of this study demonstrated that formulation F2, among the various tested formulations, exhibited favorable characteristics in terms of particle size, polydispersity index, zeta potential, drug loading, and drug release. Specifically, F2 exhibited a small particle size of 120 nm, indicative of efficient size reduction during the preparation process. This reduced size is of utmost importance, as it can facilitate improved drug delivery and the potential for enhanced penetration through biological barriers.

The low polydispersity index (PDI) of 0.15 observed in F2 indicated a narrow and homogeneous size distribution of the nanoparticles within the formulation, suggesting a more uniform and consistent drug delivery profile. Additionally, the negative zeta potential of -30 mV in F2 contributed to higher stability and better dispersion of the nanoparticles, reducing the risk of particle aggregation and ensuring long-term stability.

Moreover, F2 achieved a higher drug loading percentage of 7%, indicating efficient incorporation of Ganaxolone into the nanoparticles. This high drug loading capacity is crucial

for maximizing the therapeutic potential and efficacy of the drug, allowing for the administration of higher drug doses without increasing the nanoparticle size.

Notably, the drug release profile of F2 showed sustained and controlled release of Ganaxolone, with a release percentage of 75%. This sustained release pattern can potentially lead to prolonged drug action, reducing the dosing frequency required and enhancing patient compliance.

While the results of this study are promising, further investigations are warranted to validate the findings in more complex settings. Specifically, in vivo evaluations using animal models will be essential to assess the pharmacokinetics, bioavailability, and therapeutic efficacy of the optimized F2 formulation. These animal studies will provide valuable insights into the formulation's behavior in a physiological context, including its distribution, metabolism, and excretion.

Additionally, safety and toxicity assessments in preclinical studies will be crucial to ensure the formulation's biocompatibility and safety profile. These assessments will provide essential information on potential adverse effects and any systemic responses triggered by the nanoparticle-based drug delivery system.

Ultimately, the successful preclinical outcomes will pave the way for human clinical trials. Phase I clinical trials will assess the formulation's safety and determine the appropriate dosage range. Subsequent Phase II and Phase III trials will evaluate its efficacy in epilepsy patients, comparing the optimized nanosuspension (F2) with conventional Ganaxolone formulations or placebo controls.

In conclusion, the nanotechnology-based formulation F2 has shown promising results in terms of particle size, polydispersity index, zeta potential, drug loading, and drug release. This formulation has the potential to be a viable solution for overcoming the limitations associated with Ganaxolone and enhancing its therapeutic efficacy for the treatment of seizures. However, before this formulation can be translated into clinical practice, comprehensive in vivo studies and human clinical trials are necessary to validate its safety and efficacy. If successful, this nanosuspension could significantly contribute to the improvement of epilepsy treatment, potentially benefiting millions of patients worldwide.

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