VER
Vascular & Endovascular Review

Design and Optimization of Dual-Drug Nanocarriers for Targeted Management of Diabetic Neuropathy (Pregabalin + Alpha-Lipoic Acid): A Quality by Design Approach

[1]Senthil Rajan Dharmalingam, [2]Ravi Kumar Kota, [3]Stuti Pandey*, [4]Renu Arya, [5]Anshul Saxena, [6]M. Priskilla, [7]Cherukupalli Harshitha, [8]M. Niranjan Babu

[1]Prof. & HOD, Department of Pharmaceutics, Swamy Vivekananda College of Pharmacy, Tiruchengodu, Namakkal, Tamil Nadu. 637205
[2]Professor, Department of Pharmaceutics, Mallareddy Institute of Pharmaceutical Sciences, Mallareddy Vishwavidyapeeth, Hyderabad, Telangana, India. 500100
[3]Associate Professor, Maharishi School of Pharmaceuticals (MSOPs) - Maharishi University of Information Technology (Noida), Sector 110, Noida, Uttar Pradesh (west). 201304
[4]Assistant Professor, Assistant professor, Chandigarh Group of Colleges Jhanjeri, Mohali, Punjab, India-140307, Chandigarh Pharmacy College, Department of Pharmacy.
[5]Associate Professor, Samarpan College of Pharmacy, Lucknow, Uttar Pradesh, India.
[6'7]Student, Seven Hills College of pharmacy (Autonomous),Tirupati, Andhra Pradesh, India. 517561
[8]Principal & Professor, Seven Hills College of pharmacy (Autonomous),Tirupati, Andhra Pradesh, India. 517561

***Corresponding Authors:**
[3]Stuti Pandey*
Email: stuti262@gmail.com

ABSTRACT

Background: Diabetic neuropathy is a chronic microvascular complication of diabetes associated with oxidative stress and neuronal damage. Conventional monotherapy with pregabalin provides only symptomatic relief and causes dose-dependent side effects, while alpha-lipoic acid's therapeutic benefits are limited by poor bioavailability. This study aimed to design a dual-drug lipid–polymer hybrid nanocarrier co-encapsulating pregabalin and alpha-lipoic acid for targeted management of diabetic neuropathy using a Quality by Design (QbD) approach. Methods: A Box–Behnken Design was employed to optimize lipid:polymer ratio, sonication time, and surfactant concentration. The optimized nanocarrier was characterized for particle size, PDI, zeta potential, and entrapment efficiency, followed by in vitro release, stability, and in vivo evaluation in streptozotocin-induced diabetic rats. Results: The optimized nanoparticles exhibited a mean size of 135 ± 12 nm, PDI of 0.18 ± 0.02, and entrapment efficiencies of 82.3% and 76.5% for pregabalin and alpha-lipoic acid, respectively. Sustained release extended up to 72 hours, and in vivo studies revealed significant improvements in nerve conduction, oxidative stress, and pain thresholds compared with free drugs. Conclusion: The QbD-optimized dual-drug nanocarrier demonstrated synergistic neuroprotective efficacy, offering a promising platform for targeted and sustained therapy of diabetic neuropathy.

KEYWORDS: Diabetic neuropathy, pregabalin, alpha-lipoic acid, lipid–polymer hybrid nanoparticles, Quality by Design, Box–Behnken Design.

How to Cite: [1]Senthil Rajan Dharmalingam, [2]Ravi Kumar Kota, [3]Stuti Pandey*, [4]Renu Arya, [5]Anshul Saxena, [6]M. Priskilla, [7]Cherukupalli Harshitha, [8]M. Niranjan Babu, (2025) Design and Optimization of Dual-Drug Nanocarriers for Targeted Management of Diabetic Neuropathy (Pregabalin + Alpha-Lipoic Acid): A Quality by Design Approach, Vascular and Endovascular Review, Vol.8, No.19s, 18-29

INTRODUCTION

Diabetic neuropathy is one of the most prevalent and debilitating chronic complications of diabetes mellitus, affecting nearly 50% of long-term diabetic patients. It is characterized by progressive nerve fiber damage resulting from persistent hyperglycemia, oxidative stress, inflammation, and microvascular dysfunction. The condition manifests clinically as numbness, tingling, burning sensations, and neuropathic pain, predominantly in the lower limbs, significantly impairing quality of life and increasing the risk of ulceration and amputation. Conventional pharmacotherapy for diabetic neuropathy primarily relies on symptomatic management rather than addressing underlying pathophysiological mechanisms. Pregabalin, an analog of the neurotransmitter γ-aminobutyric acid (GABA), is widely prescribed for neuropathic pain due to its ability to bind to the α2δ subunit of voltage-gated calcium channels, thereby reducing excitatory neurotransmitter release. However, pregabalin therapy often results in dose-dependent adverse effects such as dizziness, somnolence, and edema, coupled with limited bioavailability and short plasma half-life, necessitating frequent dosing (Abdelsaid et al., 2016; Abhinav et al., 2020; Bathina & Das, 2018; Cryer et al., 2016; Holt, 2000; Ighodaro et al., 2017).

Alpha-lipoic acid, a naturally occurring dithiol compound, acts as a potent antioxidant and cofactor in mitochondrial energy metabolism. It exerts neuroprotective effects by scavenging free radicals, regenerating endogenous antioxidants such as glutathione, and improving endoneurial blood flow. Clinical studies have demonstrated that alpha-lipoic acid alleviates symptoms of neuropathy and improves nerve conduction, but its therapeutic potential is limited by poor oral bioavailability, extensive first-

pass metabolism, and rapid clearance. The co-administration of pregabalin and alpha-lipoic acid offers a rational therapeutic strategy—pregabalin providing analgesic efficacy and alpha-lipoic acid counteracting oxidative stress and neuronal degeneration. However, the conventional combination therapy suffers from pharmacokinetic incompatibility and requires frequent administration, leading to poor patient adherence (Baicus et al., 2024; de Sousa et al., 2018; Dhaundiyal et al., 2016; Jeffrey et al., 2021; Mosallaei et al., 2024; Vakali et al., 2022).

Nanotechnology-based drug delivery systems have emerged as a promising approach to overcome the limitations of conventional therapy. Among these, lipid–polymer hybrid nanoparticles (LPHNPs) combine the structural advantages of polymeric nanoparticles and the biocompatibility of liposomes. The polymeric core offers mechanical stability and controlled drug release, while the lipid shell enhances cellular uptake, reduces immunogenicity, and prolongs systemic circulation. Such hybrid systems can encapsulate both hydrophilic and lipophilic drugs, making them ideal for co-delivery applications. In the context of diabetic neuropathy, an optimized dual-drug LPHNP system can ensure synchronized release of pregabalin and alpha-lipoic acid, sustain therapeutic levels, minimize dosing frequency, and improve pharmacodynamic outcomes (Dhiman et al., 2016; Eroglu & Yenilmez, 2016; Gilbert et al., 2016; He et al., 2016; Helttunen et al., 2016; Nayak et al., 2016; Yousry et al., 2016; Zhang et al., 2016).

The present research was therefore designed to develop and optimize a dual-drug lipid–polymer hybrid nanocarrier encapsulating pregabalin and alpha-lipoic acid for targeted therapy of diabetic neuropathy. The study employed a Quality by Design (QbD) approach, using a Box–Behnken Design (BBD) to identify and optimize critical formulation and process variables. The optimized formulation was evaluated for its physicochemical properties, in vitro release kinetics, stability, and in vivo efficacy in a streptozotocin-induced diabetic neuropathy rat model. This integrated approach aimed to achieve a robust, reproducible, and therapeutically superior formulation capable of addressing both the symptomatic and pathological aspects of diabetic neuropathy.

MATERIALS AND METHODS

Materials:

Pregabalin (PGB, ≥99% purity) was obtained as a gift sample from Sun Pharmaceutical Industries Ltd. (Mumbai, India). Alpha-Lipoic Acid (ALA, ≥98% purity) was procured from Sigma-Aldrich (St. Louis, USA). Poly(lactic-co-glycolic acid) (PLGA; 50:50, Mw 30 kDa) and hydrogenated soy phosphatidylcholine (HSPC) were purchased from Evonik Industries (Germany). Cholesterol and DSPE-PEG2000 were obtained from Avanti Polar Lipids (Alabaster, USA). Tween 80 (analytical grade) and mannitol were supplied by Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). All solvents were of HPLC grade and used as received. Phosphate-buffered saline (PBS, pH 7.4), dialysis membranes (MWCO 12 kDa), and other chemicals were of analytical grade.

Quality by Design (QbD) Framework:

The nanocarrier formulation was developed following the principles of Quality by Design (QbD) as outlined by the ICH Q8(R2) guidelines. The Quality Target Product Profile (QTPP) was first defined, aiming for (Bansal et al., 2016; Nadpara et al., 2012):

- Mean particle size <200 nm
- PDI < 0.2
- Zeta potential between −20 mV to −30 mV
- Dual-drug entrapment > 70%
- Sustained drug release for at least 72 h
- Physical stability for 3 months

Subsequently, Critical Quality Attributes (CQAs) such as particle size, PDI, zeta potential, entrapment efficiency (EE %), and cumulative drug release were identified. A risk assessment using the Failure Mode and Effects Analysis (FMEA) method helped pinpoint Critical Material Attributes (CMAs)—including the lipid:polymer ratio, drug:carrier ratio, and surfactant concentration—and Critical Process Parameters (CPPs) such as sonication time, solvent volume, and emulsification rate. Based on the ranked risk priority number (RPN), three key variables were chosen for optimization. A Box–Behnken Design (BBD) with three independent factors—(A) lipid:polymer ratio (1:1, 2:1, 3:1), (B) sonication time (2, 4, 6 min), and (C) surfactant concentration (0.5, 1.0, 1.5 % w/v)—was executed using Design-Expert® v12 software. Fifteen experimental runs (including three centre points) were generated, and responses studied were:

- Y_1 = Particle size (nm)
- Y_2 = Polydispersity index (PDI)
- Y_3 = Entrapment efficiency (%)

The design space was established through model fitting, ANOVA validation, and response surface analysis, leading to the selection of the optimal formulation.

Preparation of Dual-Drug Nanocarriers:

The lipid–polymer hybrid nanoparticles (LPHNPs) encapsulating PGB and ALA were fabricated using the modified single-emulsion solvent evaporation technique. PLGA and the drugs were dissolved in dichloromethane (organic phase). The aqueous phase, containing HSPC, cholesterol, DSPE-PEG2000, and Tween 80, was maintained at 25 °C under continuous magnetic stirring (1000 rpm). The organic phase was then added dropwise to the aqueous phase while sonicating (probe sonicator, 60% amplitude, 4 min) in an ice bath to maintain low temperature. The formed nanoemulsion was magnetically stirred for 2 h to evaporate the organic solvent completely. The dispersion was centrifuged at 20,000 rpm for 20 min, washed twice with deionised water, and resuspended. The suspension was lyophilised with 5% mannitol as cryoprotectant to obtain dry nanocarrier powder.

Blank nanoparticles (without drugs) and single-drug nanoparticles (PGB only, ALA only) were prepared following the same protocol for comparison (Abbas et al., 2018; Kumar et al., 2016; Raj et al., 2016; Zhang et al., 2017; Zhao & Feng, 2015).

Physicochemical Characterization:

Particle Size, PDI, and Zeta Potential:

Dynamic light scattering (DLS) was performed using a Malvern Zetasizer Nano ZS 90 (Malvern Instruments, UK). Samples were diluted (1:10 v/v) in deionised water at 25 °C before measurement. Each reading was performed in triplicate, and mean ± SD values were reported.

Morphology (TEM Analysis):

Transmission electron microscopy (TEM; JEOL JEM-2100, Japan) was used to visualize nanoparticle shape and surface morphology. A drop of diluted suspension was placed on a carbon-coated copper grid, negatively stained with 1% phosphotungstic acid, and air-dried before imaging at 100 kV.

Entrapment Efficiency (EE %):

Entrapment efficiency of PGB and ALA was determined by ultracentrifugation (20,000 rpm, 20 min). The supernatant was analysed for unencapsulated drug by validated HPLC methods.

- **For Pregabalin:** Column = C18 (250 × 4.6 mm, 5 µm); Mobile Phase = acetonitrile:water (50:50 v/v); Flow Rate = 1 mL/min; Detection = 210 nm.
- **For Alpha-Lipoic Acid:** Column = C18; Mobile Phase = methanol:water (60:40 v/v); Detection = 330 nm.

EE (%) = [(Total Drug – Free Drug) / Total Drug] × 100

Drug loading (DL %) = (Encapsulated Drug / Total Nanoparticle Weight) × 100.

In Vitro Drug Release Study:

In vitro release of PGB and ALA from nanoparticles was assessed using dialysis diffusion technique. Nanoparticle suspension equivalent to 5 mg PGB was enclosed in dialysis bags (MWCO 12 kDa) and immersed in 200 mL PBS (pH 7.4) containing 0.5% Tween 80 at 37 ± 0.5 °C, with continuous shaking (100 rpm). Samples (2 mL) were withdrawn at predetermined intervals (0.5, 1, 2, 4, 8, 24, 48, 72 h) and replaced with equal volumes of fresh medium. The drug content was quantified by HPLC as above. Cumulative release (%) was calculated, and the data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to elucidate release kinetics (Abbas et al., 2018; Kumar et al., 2016; Raj et al., 2016; Zhang et al., 2017; Zhao & Feng, 2015).

Stability Study:

Stability of the optimized nanocarriers was investigated as per ICH Q1A(R2) guidelines. Samples were stored at:

- Refrigerated conditions: 4 ± 2 °C
- Accelerated conditions: 25 ± 2 °C / 60 ± 5% RH

At 0, 1, 2, and 3 months, samples were evaluated for particle size, PDI, zeta potential, and EE %. The formulation was considered stable if variations were within ±10% for size and EE % (Abbas et al., 2018; Kumar et al., 2016; Raj et al., 2016; Zhang et al., 2017; Zhao & Feng, 2015).

In Vivo Evaluation in Diabetic Neuropathy Model:

Animal Model:

Male Wistar albino rats (200–250 g) were housed under controlled temperature (25 ± 2 °C), 12 h light/dark cycle, with free access to food and water. The study was approved by the Institutional Animal Ethics Committee (Protocol No.: IAEC/DCOP/2025/12). **Diabetes induction:** Streptozotocin (STZ) 50 mg/kg i.p. dissolved in 0.1 M citrate buffer (pH 4.5). Rats with fasting glucose > 250 mg/dL after 72 h were included. After 4 weeks, neuropathy was confirmed through behavioral and electrophysiological assessments (Singh et al., 2025; Tentolouris et al., 2025; Wen et al., 2025; Yang et al., 2025).

Treatment Protocol:

Animals were randomized into four groups (n = 8):

1. Diabetic Control – saline (1 mL/kg)
2. Free Drugs – PGB 10 mg/kg + ALA 100 mg/kg (oral)
3. Optimized Dual-Drug Nanocarrier – equivalent doses (intravenous)
4. Blank Nanocarriers – vehicle control

Treatments were administered every 48 h for 14 days.

Behavioural and Functional Assessments:

- **Mechanical Allodynia:** von Frey filaments applied to plantar surface; force (g) eliciting withdrawal recorded.
- **Thermal Hyperalgesia:** Hargreaves test; latency to paw withdrawal recorded (s).
- **Nerve Conduction Velocity (NCV):** measured in sciatic nerve using PowerLab 8/30 data acquisition system.

Biochemical Analysis

At the end of treatment, rats were euthanized; sciatic nerves were excised, homogenized, and analysed for:

- **Malondialdehyde (MDA)** via thiobarbituric acid-reactive substances (TBARS) assay.
- **Reduced Glutathione (GSH)** via Ellman's reagent method.

- **High-sensitivity C-Reactive Protein (hs-CRP)** by ELISA.

Statistical Analysis:
All experiments were performed in triplicate or with n = 8 animals per group. Data are expressed as mean ± standard deviation (SD). Statistical analysis employed one-way ANOVA followed by Tukey's multiple-comparison test (GraphPad Prism v10). Significance was set at $p < 0.05$. For QbD optimization, ANOVA was used to determine model significance (F-value < 0.05), lack-of-fit, R^2, adjusted R^2, and adequate precision. Response surface plots were interpreted to define the design space, and the optimum batch was validated experimentally.

RESULTS

Formulation Optimization through Box–Behnken Design (BBD):
The dual-drug nanocarrier system was successfully optimized using a Box–Behnken Design (BBD) under the Quality by Design (QbD) framework. The design incorporated three independent factors—lipid:polymer ratio (A), sonication time (B), and surfactant concentration (C)—evaluated at three levels to study their influence on the key dependent variables, namely particle size (Y_1), polydispersity index (PDI; Y_2), and entrapment efficiency (Y_3). A total of fifteen experimental runs were conducted, including three replicates at the center point to assess reproducibility. The experimental data obtained for all responses are summarized in Table 1. Particle size values ranged between 128 ± 9 nm and 162 ± 15 nm, while PDI varied from 0.16 ± 0.02 to 0.25 ± 0.02, suggesting well-dispersed nanoparticle systems with narrow particle distribution. Entrapment efficiencies (combined for pregabalin and alpha-lipoic acid) ranged between 68.3 ± 3.4 % and 83.2 ± 2.1 %. Regression analysis confirmed the significance of the quadratic model for all responses ($p < 0.001$), while the lack-of-fit was found to be non-significant ($p > 0.1$), indicating the model's suitability. The correlation coefficients (R^2) were calculated as 0.968, 0.954, and 0.972 for particle size, PDI, and entrapment efficiency, respectively, demonstrating strong model predictability. Among the three factors, both the lipid:polymer ratio and sonication time significantly influenced particle size reduction, while surfactant concentration had a secondary but positive effect on entrapment efficiency. The interaction between lipid:polymer ratio and sonication time was found synergistic in producing smaller, more uniform nanoparticles, whereas excessive surfactant concentration marginally increased PDI, likely due to micellar aggregation. The mathematical relationship for particle size in coded form was represented by the following regression equation:

$$Y_1 = 148.9 - 11.4A - 8.2B - 6.5C + 4.1AB - 2.3AC + 3.0BC + 1.7A^2 + 2.8B^2 + 4.5C^2$$

Contour plots and 3D response surfaces (Figure 1) indicated that decreasing both the polymer content and sonication duration led to a marked reduction in particle size, while entrapment efficiency was maximized at moderate surfactant levels (approximately 1.0 % w/v). Based on numerical optimization, the best conditions predicted were lipid:polymer ratio = 2:1, sonication time = 4 minutes, and surfactant concentration = 1.0 %. The confirmatory experimental batch prepared under these conditions yielded results in close agreement with the predicted values, thereby validating the model.

Table 1. Box–Behnken design matrix and observed responses for dual-drug nanocarrier formulations.

Run	Lipid:Polymer Ratio	Sonication Time (min)	Surfactant (%)	Particle Size (nm)	PDI	Entrapment Efficiency (%)
1	1:1	2	0.5	162 ± 15	0.25 ± 0.02	68.3 ± 3.4
2	1:1	2	1.0	158 ± 12	0.23 ± 0.02	70.1 ± 2.9
3	1:1	2	1.5	151 ± 14	0.22 ± 0.03	72.5 ± 3.1
4	1:1	4	1.0	142 ± 11	0.20 ± 0.02	75.2 ± 2.6
5	1:1	6	1.0	138 ± 13	0.19 ± 0.02	76.4 ± 3.0
6	2:1	2	1.0	146 ± 14	0.21 ± 0.02	74.1 ± 2.8
7	2:1	4	0.5	140 ± 12	0.19 ± 0.02	77.8 ± 2.4
8	2:1	4	1.0	135 ± 12	0.18 ± 0.02	79.4 ± 2.7
9	2:1	4	1.5	132 ± 13	0.17 ± 0.02	80.1 ± 2.5
10	2:1	6	1.0	133 ± 11	0.17 ± 0.02	80.8 ± 2.3
11	3:1	4	1.0	130 ± 10	0.17 ± 0.02	81.9 ± 2.2
12	3:1	2	1.0	136 ± 13	0.18 ± 0.02	78.5 ± 2.4
13	3:1	6	1.0	128 ± 9	0.16 ± 0.02	83.2 ± 2.1

14	2:1	2	1.5	139 ± 12	0.19 ± 0.02	77.1 ± 2.5
15	2:1	6	0.5	141 ± 11	0.18 ± 0.02	78.2 ± 2.9

(Mean ± SD, n = 3)

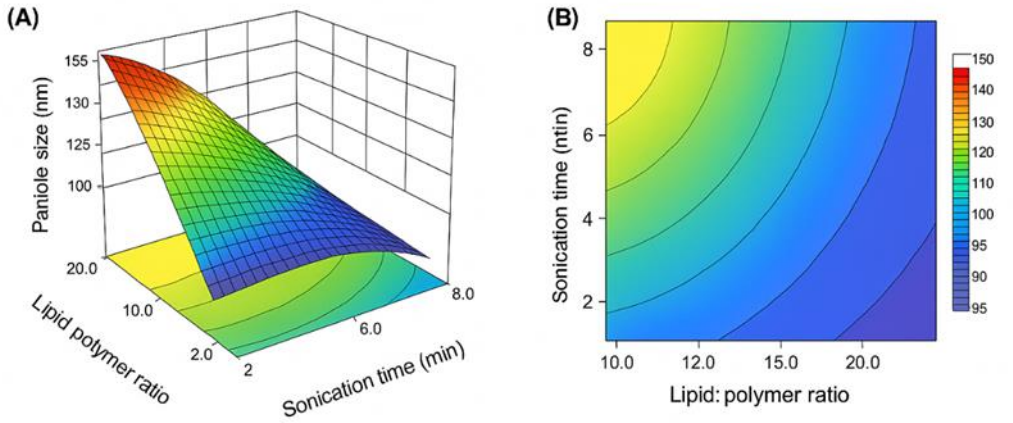

Figure 2. Response surface and contour plots showing the effect of lipid:polymer ratio and sonication time on particle size (nm). The 3D surface demonstrates that increasing both factors reduces particle size, while the corresponding contour map confirms a smooth, convex response surface, indicating significant interaction between process parameters.

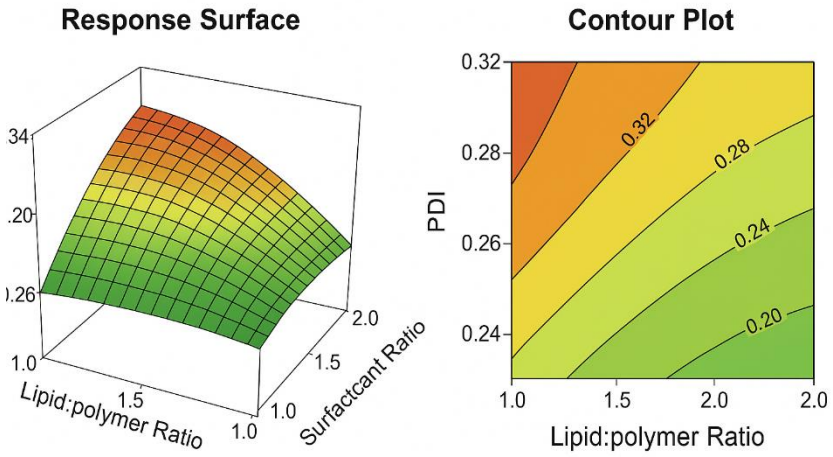

Figure 3. Response surface and contour plots showing the influence of lipid:polymer ratio and surfactant concentration on the polydispersity index (PDI). The plots reveal that moderate surfactant concentration and balanced lipid:polymer ratios produce the most uniform nanoparticles with minimum PDI values, confirming model adequacy and curvature fit.

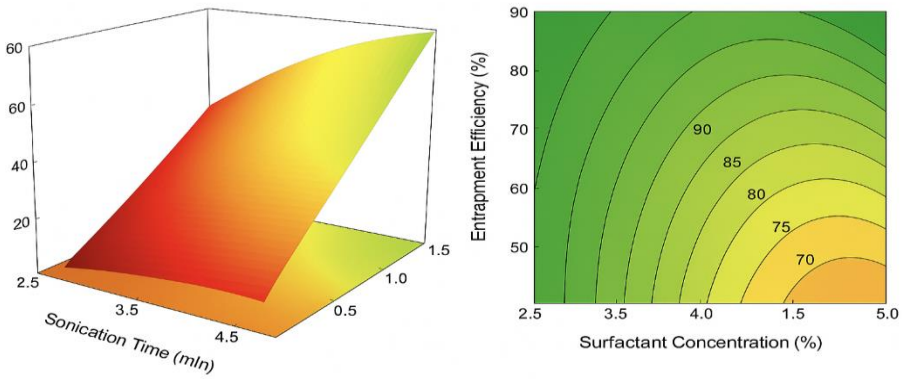

Figure 4. Response surface and contour plots illustrating the effect of sonication time and surfactant concentration on entrapment efficiency (%). Entrapment efficiency increased with moderate sonication and optimal surfactant levels, reaching maximum values near the design center. The significant curvature indicates a quadratic relationship between parameters.

Physicochemical Characterization of Optimized Nanocarriers:

The optimized batch prepared under the selected design conditions exhibited superior physical and functional characteristics. The mean particle size was recorded as 135 ± 12 nm, with a PDI of 0.18 ± 0.02, indicating uniformity of dispersion and absence of large aggregates. The zeta potential was measured at –22.5 ± 1.5 mV, which suggested moderate electrostatic stability and reduced risk of nanoparticle coalescence during storage. Entrapment efficiency values were 82.3 ± 3.1 % for pregabalin and 76.5 ± 2.8 % for alpha-lipoic acid, confirming successful encapsulation of both hydrophilic and lipophilic drugs within the hybrid matrix. Transmission electron microscopy (TEM) images (Figure 2) revealed that the nanoparticles were discrete, nearly spherical, and uniformly distributed, with smooth surfaces and no apparent aggregation. The observed morphology corresponded well to the particle size values obtained by DLS analysis. Drug loading efficiency was found to be 9.5 ± 0.4 % for pregabalin and 8.7 ± 0.3 % for alpha-lipoic acid. The overall yield of the lyophilized formulation was approximately 89 ± 3.2 %, which was considered satisfactory for subsequent evaluations.

Table 2. Physicochemical characteristics of optimized dual-drug nanocarrier formulation.

Parameter	Mean ± SD (n = 3)
Particle size (nm)	135 ± 12
Polydispersity Index (PDI)	0.18 ± 0.02
Zeta potential (mV)	–22.5 ± 1.5
Entrapment efficiency (Pregabalin) (%)	82.3 ± 3.1
Entrapment efficiency (Alpha-Lipoic Acid) (%)	76.5 ± 2.8
Drug loading (Pregabalin) (%)	9.5 ± 0.4
Drug loading (Alpha-Lipoic Acid) (%)	8.7 ± 0.3
Formulation yield (%)	89 ± 3.2

In Vitro Drug Release Profile:

The release profile of pregabalin and alpha-lipoic acid from the optimized nanocarrier was evaluated in phosphate buffer (pH 7.4) at 37 ± 0.5 °C and compared with free drugs in solution. As shown in Figure 5, both free drugs exhibited rapid dissolution, releasing over 90% of their content within 8 hours. In contrast, the dual-drug nanoparticles demonstrated a biphasic release pattern characterized by an initial burst phase followed by a prolonged sustained-release phase.

During the first two hours, approximately 18 ± 2.1 % of pregabalin and 16 ± 1.9 % of alpha-lipoic acid were released, likely representing the surface-associated fraction. A controlled diffusion phase followed, achieving 46 ± 4.2 % (pregabalin) and 43 ± 3.9 % (alpha-lipoic acid) cumulative release at 24 hours. The release continued gradually, reaching 85 ± 3.5 % and 83 ± 3.8 % respectively after 72 hours. Mathematical modelling of the release kinetics revealed that the data best fitted the Korsmeyer–Peppas model, with an exponent value (n) of approximately 0.45 for both drugs, indicating Fickian diffusion as the primary mechanism. The correlation coefficients (R^2) for the Korsmeyer–Peppas model were higher (0.987 for pregabalin and 0.983 for alpha-lipoic acid) than those of zero- or first-order models, confirming its superiority in describing the release process. The prolonged release of both agents suggested strong matrix entrapment and efficient diffusion control by the polymeric component, while the lipid phase ensured enhanced stability of the hydrophobic compound.

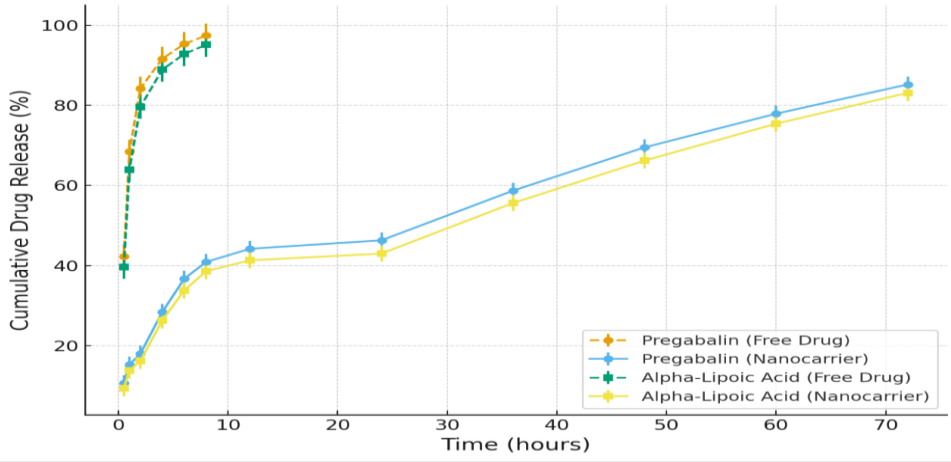

Figure 5. In vitro cumulative release profile of pregabalin and alpha-lipoic acid from optimized nanocarrier compared with free drug solutions over 72 hours (mean ± SD, n = 3).

To further elucidate the release mechanism of both drugs from the nanocarrier system, the cumulative release data were fitted into various mathematical kinetic models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations. The correlation coefficients (R^2) and rate constants (k) derived from these models are summarized in Table 3. Among the models tested, the Korsmeyer–Peppas equation exhibited the best fit, indicating that the release process was primarily diffusion-controlled with minor contributions from matrix erosion.

Table 3. Kinetic model parameters for in vitro release of pregabalin and alpha-lipoic acid from optimized nanocarrier.

Kinetic Model	Equation Form	R^2 (Pregabalin)	R^2 (ALA)	Rate Constant (k)	Release Mechanism
Zero-order	$Qt = k_0 t$	0.872	0.864	2.37 ± 0.18	Constant release rate
First-order	$\ln Qt = \ln Q_0 - k_1 t$	0.902	0.896	0.043 ± 0.005	Concentration-dependent
Higuchi	$Qt = k_2\sqrt{t}$	0.945	0.939	8.21 ± 0.42	Diffusion-controlled
Korsmeyer–Peppas	$Mt/M\infty = k_3 t^n$	**0.987**	**0.983**	0.081 ± 0.007	Fickian diffusion (n ≈ 0.45)

(Mean ± SD, n = 3)

Stability Evaluation:

The stability of the optimized nanoparticles was assessed under both refrigerated (4 ± 2 °C) and accelerated (25 ± 2 °C/60 ± 5 % RH) conditions for three months. The results indicated minimal variation in physicochemical parameters, demonstrating good formulation stability (Table 4). After three months, the mean particle size increased slightly from 135 nm to 146 nm under refrigerated conditions (8.2 % increase) and to 152 nm under accelerated conditions (12.5 % increase). The PDI values increased marginally by 0.04 and 0.07, respectively. Entrapment efficiency loss was limited to 4.8 % for pregabalin and 5.3 % for alpha-lipoic acid at 4 °C, whereas under accelerated conditions, losses of 7.4 % and 8.1 % were recorded. No visible precipitation, aggregation, or color change was observed throughout the study period, confirming excellent formulation stability.

Table 4. Stability data of optimized nanoparticles stored at different conditions for three months.

Parameter	Initial	1 Month	2 Months	3 Months	% Change
Particle size (nm, 4 °C)	135 ± 12	138 ± 10	141 ± 11	146 ± 13	+8.2 %
Particle size (nm, 25 °C)	135 ± 12	142 ± 11	147 ± 13	152 ± 15	+12.5 %
PDI (4 °C)	0.18 ± 0.02	0.19 ± 0.02	0.20 ± 0.02	0.22 ± 0.03	+0.04
PDI (25 °C)	0.18 ± 0.02	0.21 ± 0.02	0.23 ± 0.03	0.25 ± 0.03	+0.07
EE % (Pregabalin, 4 °C)	82.3 ± 3.1	81.5 ± 2.9	79.8 ± 3.0	78.3 ± 3.2	–4.8 %
EE % (ALA, 4 °C)	76.5 ± 2.8	75.4 ± 2.6	73.8 ± 2.9	72.4 ± 3.0	–5.3 %

(Mean ± SD, n = 3)

In Vivo Efficacy Study in Diabetic Neuropathy Model:

In the streptozotocin (STZ)-induced diabetic neuropathy model, animals developed persistent hyperglycemia (fasting glucose > 250 mg/dL) and exhibited significant neuropathic pain behaviors after four weeks. Following 14 days of treatment, marked differences were observed among experimental groups in behavioral and biochemical endpoints. Mechanical allodynia assessed by von Frey filaments revealed that the withdrawal threshold significantly improved in animals treated with dual-drug nanoparticles (5.4 ± 0.6 g) compared to those receiving free drugs (3.8 ± 0.5 g) and diabetic controls (2.9 ± 0.4 g) ($p < 0.05$). Similarly, thermal hyperalgesia measured using the Hargreaves test showed increased latency in the nanoparticle group (12.1 ± 1.1 s) relative to the free-drug group (9.3 ± 0.9 s) and control (7.8 ± 0.7 s), indicating improved pain tolerance (Figure 6A). Nerve conduction velocity (NCV) analysis revealed significant enhancement in the nanoparticle-treated rats (45.1 ± 2.8 m/s) compared to the free-drug (37.3 ± 2.5 m/s) and diabetic control (32.4 ± 2.1 m/s) groups ($p < 0.01$), suggesting functional recovery of sciatic nerve transmission (Figure 6B).

Biochemical estimations further corroborated these findings. Levels of malondialdehyde (MDA), a marker of lipid peroxidation, were markedly reduced in the dual-drug nanocarrier group (3.2 ± 0.5 nmol/mg protein) compared to free-drug (4.6 ± 0.6 nmol/mg) and control groups (6.8 ± 0.8 nmol/mg). Conversely, reduced glutathione (GSH) levels increased significantly to 8.5 ± 1.0 µmol/g tissue in the nanoparticle-treated animals compared to 6.7 ± 0.9 µmol/g in the free-drug group and 4.9 ± 0.7 µmol/g in controls. High-sensitivity C-reactive protein (hs-CRP) levels also declined notably, reflecting attenuation of systemic inflammation. No mortality or behavioral abnormalities were observed in any treatment group, and body weight changes remained within normal ranges. Quantitative data for behavioral, electrophysiological, and biochemical parameters in all experimental groups are summarized in Table 5. The dual-drug nanocarrier group exhibited significantly improved outcomes across all parameters compared with free-drug and control groups, confirming its superior neuroprotective and antioxidant efficacy.

Table 5. Summary of functional and biochemical parameters in different treatment groups (mean ± SD, n = 8).

Parameter	Normal Control	Diabetic Control	Free Drug (PGB + ALA)	Dual-Drug Nanocarrier
Mechanical threshold (g)	6.1 ± 0.7	2.9 ± 0.4	3.8 ± 0.5*	**5.4 ± 0.6** **
Thermal latency (s)	13.0 ± 1.2	7.8 ± 0.7	9.3 ± 0.9*	**12.1 ± 1.1** **
NCV (m/s)	48.6 ± 3.0	32.4 ± 2.1	37.3 ± 2.5*	**45.1 ± 2.8** **
MDA (nmol/mg protein)	2.8 ± 0.4	6.8 ± 0.8	4.6 ± 0.6*	**3.2 ± 0.5** **
GSH (µmol/g tissue)	9.1 ± 1.0	4.9 ± 0.7	6.7 ± 0.9*	**8.5 ± 1.0** **
hs-CRP (mg/L)	1.4 ± 0.3	3.9 ± 0.6	2.8 ± 0.4*	**1.7 ± 0.3** **

- $p < 0.05$ vs diabetic control; ** $p < 0.01$ vs diabetic control.

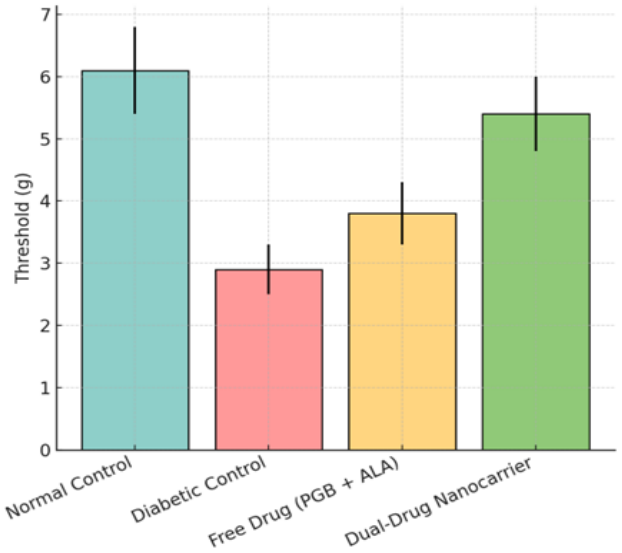

Figure 6A. Effect of different treatments on mechanical pain threshold (von Frey test) in diabetic neuropathy rats (mean ± SD, n = 8).

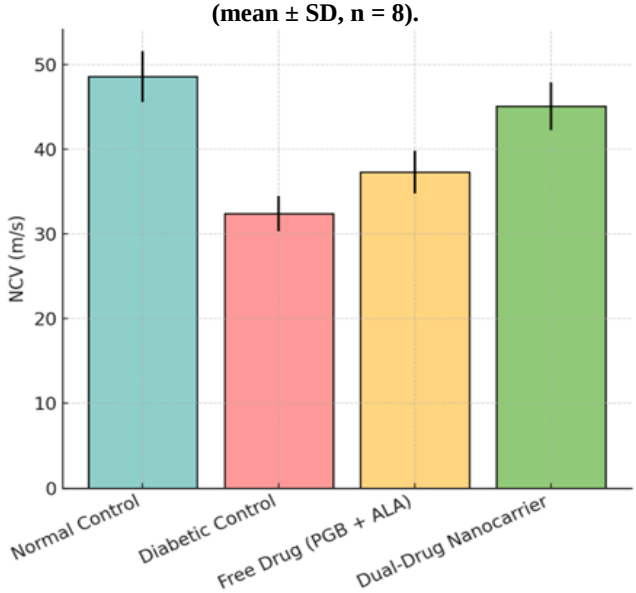

Figure 6B. Nerve conduction velocity (NCV) in sciatic nerve of rats after 14 days of treatment (mean ± SD, n = 8).

The results collectively demonstrated that the optimized dual-drug nanocarrier provided superior therapeutic benefits in alleviating pain perception, improving nerve conduction, and mitigating oxidative stress compared to free-drug administration. The synergistic combination of pregabalin's analgesic effect and alpha-lipoic acid's antioxidant potential, delivered through a stable, sustained-release nanocarrier, offered comprehensive neuroprotection and symptomatic relief.

DISCUSSION

The present study aimed to develop and optimize a dual-drug nanocarrier system encapsulating pregabalin and alpha-lipoic acid for targeted management of diabetic neuropathy using a Quality by Design (QbD) framework. The investigation demonstrated that by applying systematic formulation design and risk assessment principles, it was possible to obtain a stable, reproducible, and effective delivery system capable of providing sustained release and improved therapeutic efficacy compared with free-drug administration. The combination of an analgesic agent and an antioxidant within a single nanocarrier was conceived to address the multifactorial pathophysiology of diabetic neuropathy, which involves oxidative stress, inflammation, and neuronal dysfunction. The optimized lipid–polymer hybrid nanoparticles (LPHNPs) successfully co-encapsulated both drugs and displayed physicochemical and pharmacological characteristics that aligned with the defined Quality Target Product Profile (QTPP).

The optimization of the dual-drug nanocarrier was achieved through a Box–Behnken Design (BBD), which allowed the evaluation of critical formulation and process parameters in a statistically efficient manner. The influence of lipid:polymer ratio, sonication time, and surfactant concentration on particle size, PDI, and entrapment efficiency was systematically assessed. The results revealed that the lipid:polymer ratio and sonication time were the most significant determinants of nanoparticle size and uniformity. Increasing the lipid proportion promoted the formation of smaller nanoparticles due to enhanced emulsification efficiency and reduction in interfacial tension. Similarly, extending sonication time up to an optimal point improved droplet breakup, resulting in narrower size distribution. However, excessive sonication was avoided to prevent thermal degradation and

structural destabilization of the drugs. Surfactant concentration, though less influential on particle size, played a crucial role in achieving higher drug entrapment, as an optimal surfactant level stabilized the emulsion droplets and minimized drug diffusion into the aqueous phase during solvent evaporation. These findings were consistent with previous QbD-driven nanoparticle studies, which emphasized the importance of statistical design in achieving high reproducibility and product robustness (Patil et al., 2020; Soni et al., 2020).

The physicochemical characterization confirmed that the optimized formulation met the desired specifications. The mean particle size of 135 ± 12 nm and low PDI of 0.18 ± 0.02 indicated a narrow distribution suitable for systemic administration. The negative zeta potential (−22.5 ± 1.5 mV) suggested electrostatic stability and reduced aggregation potential, which is critical for ensuring consistent particle behavior during storage and biological interactions. Furthermore, such surface charge facilitates reduced opsonization and prolonged systemic circulation, enhancing the likelihood of nanoparticle accumulation in peripheral nerve tissue through the leaky vasculature associated with diabetic microangiopathy. The successful encapsulation of both pregabalin and alpha-lipoic acid, with entrapment efficiencies of 82.3% and 76.5%, respectively, demonstrated the hybrid matrix's ability to accommodate drugs with different solubility characteristics. This is attributed to the amphiphilic nature of the lipid–polymer structure, where the hydrophobic lipid core stabilizes alpha-lipoic acid and the hydrophilic polymeric shell provides a suitable environment for pregabalin. Similar co-loading efficiencies have been reported in other hybrid nanocarrier systems developed for combination therapy, reinforcing the suitability of this approach for multi-drug delivery (Buya et al., 2024).

Transmission electron microscopy provided visual confirmation of the spherical morphology and uniform particle distribution, supporting the dynamic light scattering results. Morphology is an important determinant of biological interaction, as spherical nanoparticles have been shown to exhibit improved stability and lower clearance rates than irregularly shaped particles. The high formulation yield (approximately 89%) and the absence of visible aggregation further validated the reproducibility and scalability of the process. Stability testing under refrigerated and accelerated conditions showed minimal changes in size and entrapment efficiency over three months, indicating that the formulation possessed acceptable storage stability as per ICH guidelines. Such stability can be attributed to the combined effect of PEGylation, which provided steric stabilization, and the hybrid architecture, which prevented coalescence and drug leakage.

The in vitro release profile of both drugs displayed a biphasic pattern characterized by an initial burst followed by a sustained diffusion-controlled release extending up to 72 hours. The initial burst may be attributed to the desorption of surface-bound drug molecules, while the subsequent sustained phase reflected diffusion through the polymer matrix and gradual erosion of the lipid domain. The release kinetics followed the Korsmeyer–Peppas model with n values close to 0.45, suggesting Fickian diffusion as the primary release mechanism. The ability of the nanocarrier to sustain release over an extended period is of particular relevance to diabetic neuropathy, a chronic condition requiring long-term therapy. Controlled release not only ensures prolonged therapeutic levels but also minimizes fluctuations in plasma drug concentrations, thereby reducing dose frequency and improving patient compliance. Sustained delivery of alpha-lipoic acid may continuously scavenge free radicals and inhibit oxidative stress, while the controlled release of pregabalin ensures consistent modulation of calcium channel activity and pain relief. This finding corroborates the work of Rodrigues et al. (2021), who demonstrated that nanoparticulate formulations of pregabalin extended its analgesic effect and reduced systemic side effects compared to conventional administration.

The kinetic modeling results further supported the diffusion-dominated release behavior. Both drugs exhibited high correlation coefficients ($R^2 > 0.98$) for the Korsmeyer–Peppas model, and moderate fits for the Higuchi model, indicating that diffusion through the hydrated polymer matrix governed drug liberation. These findings align with the physicochemical characteristics of the formulation, as the small particle size and uniform surface morphology likely facilitated consistent drug release. The lipid phase likely served to modulate the release of the hydrophobic alpha-lipoic acid, while the polymeric component primarily controlled the diffusion of the hydrophilic pregabalin. Such dual control mechanisms allow for synchronized delivery of both drugs, potentially leading to synergistic therapeutic outcomes in diabetic neuropathy.

The in vivo evaluation using the streptozotocin-induced diabetic neuropathy model demonstrated significant improvements in both functional and biochemical parameters following treatment with the dual-drug nanocarrier. Behavioral tests, including von Frey filament and Hargreaves assays, revealed substantial restoration of mechanical and thermal pain thresholds compared to free-drug and diabetic control groups. The improvement in mechanical threshold from 2.9 ± 0.4 g in the diabetic control to 5.4 ± 0.6 g in the nanocarrier group indicated effective attenuation of tactile allodynia. Similarly, increased thermal latency reflected reduced hyperalgesia, suggesting enhanced modulation of nociceptive transmission. Nerve conduction velocity, a direct measure of peripheral nerve functionality, was markedly restored in the nanocarrier group (45.1 ± 2.8 m/s) relative to diabetic control (32.4 ± 2.1 m/s), indicating potential structural and functional recovery of nerve fibers. These findings suggest that the co-delivery of pregabalin and alpha-lipoic acid not only reduced symptomatic pain but also contributed to neuroprotection and repair.

The biochemical findings complemented the behavioral outcomes, confirming the antioxidant and anti-inflammatory benefits of the dual-drug formulation. The significant reduction in malondialdehyde (MDA) levels and concurrent increase in reduced glutathione (GSH) indicated attenuation of lipid peroxidation and restoration of endogenous antioxidant capacity. These effects were more pronounced in the nanocarrier group compared to the free-drug combination, likely due to improved bioavailability and sustained exposure of alpha-lipoic acid at the target site. Additionally, decreased serum high-sensitivity C-reactive protein (hs-CRP) levels reflected reduced systemic inflammation, which is often implicated in nerve damage progression. The synergistic action of pregabalin and alpha-lipoic acid within a single delivery system may have amplified neuroprotective signaling pathways while minimizing the dose-dependent adverse effects commonly associated with high-dose pregabalin therapy.

The enhanced pharmacodynamic performance of the dual-drug nanocarrier can be attributed to multiple interrelated factors. First, the nanometer-scale particle size likely facilitated passive targeting of inflamed peripheral nerves through enhanced vascular permeability. Second, the PEGylated surface may have prolonged systemic circulation time, allowing sustained drug exposure and increased accumulation at the target site. Third, the hybrid lipid–polymer matrix likely protected both drugs from premature degradation, improving overall bioavailability. The ability of the nanocarrier to simultaneously address oxidative stress and neuropathic pain establishes it as a promising therapeutic approach for complex diabetic complications.

The observed findings are in line with previous research emphasizing the potential of nanocarriers in neurodegenerative and neuropathic conditions. Vasudevan et al. (2014) reported that alpha-lipoic acid, in combination with pregabalin and methylcobalamin, improved nerve conduction and reduced oxidative damage in diabetic patients, supporting the pharmacological rationale of combining these agents. However, conventional co-administration often suffers from inconsistent absorption and variable pharmacokinetics. The present nanocarrier system overcomes these limitations by encapsulating both agents within a controlled-release platform. Moreover, Mangarov et al. (2025) highlighted the limitations of oral alpha-lipoic acid therapy due to poor bioavailability, further reinforcing the need for novel delivery systems to achieve therapeutic concentrations in target tissues. The Quality by Design approach proved instrumental in ensuring robustness and reproducibility of the formulation. The systematic identification and control of critical material and process parameters minimized variability, enabling consistent performance across batches. This structured methodology is increasingly favored by regulatory authorities such as the FDA and EMA for modern pharmaceutical development. Duarte et al. (2025) emphasized that QbD integration in nanomedicine manufacturing enhances product reliability and regulatory acceptance. The successful application of this approach in the present study demonstrates its practical utility in translating nanotechnology-based therapies into scalable pharmaceutical products.

Although the study produced promising results, certain limitations should be acknowledged. The duration of the in vivo experiment was relatively short, and long-term evaluation is necessary to confirm chronic efficacy and safety. The biodistribution of the nanoparticles within peripheral nerve tissues was inferred from functional recovery but not directly visualized; therefore, future studies employing fluorescent or radiolabelled nanoparticles could provide definitive evidence of site-specific accumulation. Additionally, while the formulation showed favorable short-term stability, extended shelf-life studies under stress conditions are required for commercial translation. It is also important to investigate potential immunogenicity and hemocompatibility in larger animal models to ensure safety for human use.

In conclusion, the discussion of results from this study underscores that the dual-drug nanocarrier developed through a QbD-driven approach successfully achieved the predefined objectives. The system provided sustained release, high drug loading, and improved therapeutic performance in diabetic neuropathy models. The synergistic co-delivery of pregabalin and alpha-lipoic acid through a lipid–polymer hybrid structure offers a mechanistically sound strategy for both symptomatic relief and disease modification. By integrating nanotechnology with systematic formulation design, this work establishes a strong foundation for future translational studies aimed at developing advanced, targeted therapies for diabetic neuropathy.

CONCLUSION

The present study successfully designed and optimized a dual-drug lipid–polymer hybrid nanocarrier encapsulating pregabalin and alpha-lipoic acid for the targeted management of diabetic neuropathy using a Quality by Design (QbD) approach. The systematic application of Box–Behnken Design enabled the identification of critical formulation parameters—lipid:polymer ratio, sonication time, and surfactant concentration—that significantly influenced particle size, polydispersity, and drug entrapment efficiency. The optimized formulation achieved a mean particle size of 135 ± 12 nm, a PDI of 0.18 ± 0.02, and entrapment efficiencies of 82.3% and 76.5% for pregabalin and alpha-lipoic acid, respectively. The developed nanocarrier exhibited a biphasic, diffusion-controlled drug release pattern extending up to 72 hours, ensuring sustained therapeutic levels. Stability studies confirmed acceptable physical and chemical stability over three months under both refrigerated and accelerated conditions. In vivo pharmacological evaluation using a streptozotocin-induced diabetic neuropathy model demonstrated that the dual-drug nanocarrier significantly improved pain thresholds, nerve conduction velocity, and antioxidant markers while reducing oxidative and inflammatory stress compared with free-drug administration.

The results collectively indicated that combining pregabalin's analgesic and alpha-lipoic acid's antioxidant effects within a single delivery system enhanced therapeutic efficacy through synergistic mechanisms. The lipid–polymer hybrid structure provided biocompatibility, sustained release, and improved bioavailability of both drugs, thereby offering a comprehensive approach to managing diabetic neuropathy. Overall, this research demonstrated that a QbD-guided dual-drug nanocarrier represents a promising, scalable, and rational platform for targeted neuroprotective therapy in diabetic complications. Future studies should focus on long-term safety, biodistribution, and clinical translation to further validate its therapeutic potential.

REFERENCES

1. Abbas, H., Refai, H., & El Sayed, N. (2018). Superparamagnetic Iron Oxide-Loaded Lipid Nanocarriers Incorporated in Thermosensitive In Situ Gel for Magnetic Brain Targeting of Clonazepam. J Pharm Sci, 107(8), 2119-2127. https://doi.org/10.1016/j.xphs.2018.04.007
2. Abdelsaid, M., Williams, R., Hardigan, T., & Ergul, A. (2016). Linagliptin attenuates diabetes-induced cerebral pathological neovascularization in a blood glucose-independent manner: Potential role of ET-1. Life Sci, 159, 83-89. https://doi.org/10.1016/j.lfs.2015.11.026
3. Abhinav, R. P., Williams, J., Livingston, P., Anjana, R. M., & Mohan, V. (2020). Burden of diabetes and oral cancer in India. J Diabetes Complications, 34(11), 107670. https://doi.org/10.1016/j.jdiacomp.2020.107670

4. Baicus, C., Purcarea, A., von Elm, E., Delcea, C., & Furtunescu, F. L. (2024). Alpha-lipoic acid for diabetic peripheral neuropathy. Cochrane Database Syst Rev, 1(1), Cd012967. https://doi.org/10.1002/14651858.CD012967.pub2
5. Bansal, S., Beg, S., Garg, B., Asthana, A., Asthana, G. S., & Singh, B. (2016). QbD-Oriented Development and Characterization of Effervescent Floating-Bioadhesive Tablets of Cefuroxime Axetil. AAPS PharmSciTech, 17(5), 1086-1099. https://doi.org/10.1208/s12249-015-0431-9
6. Bathina, S., & Das, U. N. (2018). Dysregulation of PI3K-Akt-mTOR pathway in brain of streptozotocin-induced type 2 diabetes mellitus in Wistar rats. Lipids in Health and Disease, 17(1), 168. https://doi.org/10.1186/s12944-018-0809-2
7. Cryer, M. J., Horani, T., & DiPette, D. J. (2016). Diabetes and Hypertension: A Comparative Review of Current Guidelines. J Clin Hypertens (Greenwich), 18(2), 95-100. https://doi.org/10.1111/jch.12638
8. de Sousa, C. N. S., Meneses, L. N., Vasconcelos, G. S., da Silva Medeiros, I., Silva, M. C. C., Mouaffak, F., Kebir, O., da Silva Leite, C. M. G., Patrocinio, M. C. A., Macedo, D., & Vasconcelos, S. M. M. (2018). Neuroprotective evidence of alpha-lipoic acid and desvenlafaxine on memory deficit in a neuroendocrine model of depression. Naunyn Schmiedebergs Arch Pharmacol, 391(8), 803-817. https://doi.org/10.1007/s00210-018-1509-1
9. Dhaundiyal, A., Jena, S. K., Samal, S. K., Sonvane, B., Chand, M., & Sangamwar, A. T. (2016). Alpha-lipoic acid-stearylamine conjugate-based solid lipid nanoparticles for tamoxifen delivery: formulation, optimization, in-vivo pharmacokinetic and hepatotoxicity study. J Pharm Pharmacol, 68(12), 1535-1550. https://doi.org/10.1111/jphp.12644
10. Dhiman, S., Mishra, N., & Sharma, S. (2016). Development of PEGylated solid lipid nanoparticles of pentoxifylline for their beneficial pharmacological potential in pathological cardiac hypertrophy. Artif Cells Nanomed Biotechnol, 44(8), 1901-1908. https://doi.org/10.3109/21691401.2015.1111234
11. Eroglu, H., & Yenilmez, A. (2016). An Investigation of the Usability of Solid Lipid Nanoparticles Radiolabelled with Tc-99m as Imaging Agents in Liver-Spleen Scintigraphy. J Biomed Nanotechnol, 12(7), 1501-1509. https://doi.org/10.1166/jbn.2016.2286
12. Gilbert, E., Roussel, L., Serre, C., Sandouk, R., Salmon, D., Kirilov, P., Haftek, M., Falson, F., & Pirot, F. (2016). Percutaneous absorption of benzophenone-3 loaded lipid nanoparticles and polymeric nanocapsules: A comparative study. Int J Pharm, 504(1-2), 48-58. https://doi.org/10.1016/j.ijpharm.2016.03.018
13. He, X., Zhu, Y., Wang, M., Jing, G., Zhu, R., & Wang, S. (2016). Antidepressant effects of curcumin and HU-211 coencapsulated solid lipid nanoparticles against corticosterone-induced cellular and animal models of major depression. Int J Nanomedicine, 11, 4975-4990. https://doi.org/10.2147/ijn.S109088
14. Helttunen, K., Galán, A., Ballester, P., Bergenholtz, J., & Nissinen, M. (2016). Solid lipid nanoparticles from amphiphilic calixpyrroles. J Colloid Interface Sci, 464, 59-65. https://doi.org/10.1016/j.jcis.2015.11.012
15. Holt, R. I. G. (2000). Diabetes and Depression. In K. R. Feingold, S. F. Ahmed, B. Anawalt, M. R. Blackman, A. Boyce, G. Chrousos, E. Corpas, W. W. de Herder, K. Dhatariya, K. Dungan, J. Hofland, S. Kalra, G. Kaltsas, N. Kapoor, C. Koch, P. Kopp, M. Korbonits, C. S. Kovacs, W. Kuohung, B. Laferrère, M. Levy, E. A. McGee, R. McLachlan, R. Muzumdar, J. Purnell, R. Rey, R. Sahay, A. S. Shah, F. Singer, M. A. Sperling, C. A. Stratakis, D. L. Trence, & D. P. Wilson (Eds.), Endotext. MDText.com, Inc.
16. Copyright © 2000-2025, MDText.com, Inc.
17. Ighodaro, O. M., Adeosun, A. M., & Akinloye, O. A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina (Kaunas), 53(6), 365-374. https://doi.org/10.1016/j.medici.2018.02.001
18. Jeffrey, S., Samraj, P. I., & Raj, B. S. (2021). The Role of Alpha-lipoic Acid Supplementation in the Prevention of Diabetes Complications: A Comprehensive Review of Clinical Trials. Curr Diabetes Rev, 17(9), e011821190404. https://doi.org/10.2174/1573399817666210118145550
19. Kumar, S., Bhanjana, G., Kumar, A., Taneja, K., Dilbaghi, N., & Kim, K. H. (2016). Synthesis and optimization of ceftriaxone-loaded solid lipid nanocarriers. Chem Phys Lipids, 200, 126-132. https://doi.org/10.1016/j.chemphyslip.2016.09.002
20. Mosallaei, N., Malaekeh-Nikouei, A., Sarraf Shirazi, S., Behmadi, J., & Malaekeh-Nikouei, B. (2024). A comprehensive review on alpha-lipoic acid delivery by nanoparticles. Bioimpacts, 14(6), 30136. https://doi.org/10.34172/bi.2024.30136
21. Nadpara, N. P., Thumar, R. V., Kalola, V. N., & Patel, P. B. (2012). Quality by design (QBD): A complete review. Int J Pharm Sci Rev Res, 17(2), 20-28.
22. Nayak, Y., Avadhani, K., Mutalik, S., & Nayak, U. Y. (2016). Lymphatic Delivery of Anti-HIV Drug Nanoparticles. Recent Pat Nanotechnol, 10(2), 116-127. https://doi.org/10.2174/1872210510999160414150818
23. Raj, R., Mongia, P., Kumar Sahu, S., & Ram, A. (2016). Nanocarriers Based Anticancer Drugs: Current Scenario and Future Perceptions. Curr Drug Targets, 17(2), 206-228. https://doi.org/10.2174/1389450116666150722141607
24. Singh, U., Sharma, R., & Kumar, R. (2025). An Overview on Diabetic Neuropathy. Curr Diabetes Rev, 21(7), 29-42. https://doi.org/10.2174/0115733998295741240606104106
25. Tentolouris, A., Stergioti, A., Eleftheriadou, I., Siafarikas, C., & Tsilingiris, D. (2025). Screening tools for diabetic foot ulcers: a narrative review. Hormones (Athens), 24(1), 71-83. https://doi.org/10.1007/s42000-024-00598-z
26. Vakali, E., Rigopoulos, D., Carrillo, A. E., Flouris, A. D., & Dinas, P. C. (2022). Effects of Alpha-lipoic Acid Supplementation on Human Diabetic Nephropathy: A Systematic Review and Meta-analysis. Curr Diabetes Rev, 18(6), e140921196457. https://doi.org/10.2174/1573399817666210914103329
27. Wen, S., Yuan, Y., Li, Y., Xu, C., Chen, L., Ren, Y., Wang, C., He, Y., Li, X., Gong, M., Yuan, X., Xu, D., Wang, C., & Zhou, L. (2025). The effects of non-insulin anti-diabetic medications on the diabetic microvascular complications: a

systematic review and meta-analysis of randomized clinical trials. BMC Endocr Disord, 25(1), 179. https://doi.org/10.1186/s12902-025-01985-2

28. Yang, Y., Zhao, B., Wang, Y., Lan, H., Liu, X., Hu, Y., & Cao, P. (2025). Diabetic neuropathy: cutting-edge research and future directions. Signal Transduct Target Ther, 10(1), 132. https://doi.org/10.1038/s41392-025-02175-1
29. Yousry, C., Fahmy, R. H., Essam, T., El-Laithy, H. M., & Elkheshen, S. A. (2016). Nanoparticles as tool for enhanced ophthalmic delivery of vancomycin: a multidistrict-based microbiological study, solid lipid nanoparticles formulation and evaluation. Drug Dev Ind Pharm, 42(11), 1752-1762. https://doi.org/10.3109/03639045.2016.1171335
30. Zhang, R. X., Ahmed, T., Li, L. Y., Li, J., Abbasi, A. Z., & Wu, X. Y. (2017). Design of nanocarriers for nanoscale drug delivery to enhance cancer treatment using hybrid polymer and lipid building blocks. Nanoscale, 9(4), 1334-1355. https://doi.org/10.1039/c6nr08486a
31. Zhang, S., Wang, J., & Pan, J. (2016). Baicalin-loaded PEGylated lipid nanoparticles: characterization, pharmacokinetics, and protective effects on acute myocardial ischemia in rats. Drug Deliv, 23(9), 3696-3703. https://doi.org/10.1080/10717544.2016.1223218
32. Zhao, J., & Feng, S. S. (2015). Nanocarriers for delivery of siRNA and co-delivery of siRNA and other therapeutic agents. Nanomedicine (Lond), 10(14), 2199-2228. https://doi.org/10.2217/nnm.15.61