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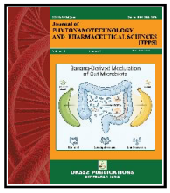

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Development, optimisation, and *in vitro* evaluation of nanoengineered etoposide-loaded polymeric nanoparticles for improved anticancer therapy

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Abstract

Etoposide is a widely used chemotherapeutic agent whose clinical application is limited by poor aqueous solubility, non-specific biodistribution, and dose-dependent systemic toxicity. Nanoengineered drug delivery systems offer a promising strategy to overcome these limitations by enabling controlled drug release and enhanced tumour-specific cytotoxicity. In the present study, etoposide-loaded polymeric nanoparticles were developed using a PLGA-based emulsification-solvent evaporation technique and optimised through a quality by design approach employing a box-behnken experimental design. The optimised formulation was characterised for particle size, surface charge, entrapment efficiency, drug loading, and *in vitro* release behaviour. Stability studies were conducted under refrigerated and accelerated conditions. Anticancer efficacy was evaluated using MTT cytotoxicity assays, cellular uptake analysis, and annexin V-FITC/propidium iodide-based apoptosis assessment in MCF-7 and HeLa cancer cell lines. The optimised nanoformulation exhibited a nanoscale particle size with narrow size distribution, high drug entrapment efficiency, and sustained drug release following diffusion-controlled kinetics. Stability studies confirmed minimal physicochemical variation over three months. *In vitro* cytotoxicity studies demonstrated significantly reduced IC_{50} values for nanoencapsulated etoposide compared to the free drug, while blank nanoparticles showed negligible toxicity. Apoptosis analysis revealed markedly enhanced early and late apoptotic cell populations following nanoparticle treatment, confirming superior intracellular delivery and prolonged anticancer activity. Nanoengineered etoposide-loaded PLGA nanoparticles effectively enhanced controlled cytotoxicity and anticancer efficacy, highlighting their potential as an advanced delivery platform for improving the therapeutic index of etoposide.

1. Introduction

Cancer remains a leading cause of morbidity and mortality worldwide, with chemotherapy continuing to play a central role in the management of a wide range of malignancies. Despite significant advances in targeted therapies and immuno-oncology, conventional cytotoxic agents remain indispensable, particularly in solid tumours and haematological cancers. Etoposide, a semi-synthetic derivative of podophyllotoxin, has been extensively employed in the treatment of lung cancer, testicular cancer, lymphomas, and leukaemias owing to its potent inhibition of DNA topoisomerase II. However, the clinical utility of etoposide has been substantially constrained by its poor aqueous solubility, erratic bioavailability, and severe dose-related toxicities, including myelosuppression and secondary malignancies (Xie *et al.,* 2025; Yi *et al.,* 2025; You and Zhang, 2025; Zhang *et al.,* 2025). Conventional etoposide formulations rely on high systemic dosing to achieve therapeutic concentrations at tumour sites, leading to non-specific distribution and damage to healthy tissues. Rapid plasma clearance and limited tumour retention further compromise therapeutic efficacy, often necessitating repeated dosing cycles that exacerbate adverse effects. These limitations underscore the need for advanced delivery strategies capable of improving drug solubility, prolonging systemic circulation, and enhancing tumour-specific accumulation while minimising off-target toxicity (You and Zhang, 2025; Zhang *et al.,* 2025; Zhang *et al.,* 2025; Zhou *et al.,* 2025).

Nanotechnology-based drug delivery systems have emerged as a powerful approach to address these challenges. Polymeric nanoparticles, particularly those fabricated from poly (lactic-co-glycolic acid), have attracted considerable attention due to their biodegradability, biocompatibility, and regulatory acceptance. Such systems offer the ability to encapsulate hydrophobic drugs, protect them from premature degradation, and provide controlled and sustained release profiles (Sreeharsha *et al.,* 2025; Tiwary *et al.,* 2025; Yilmaz *et al.,* 2025; Zaheer *et al.,* 2025; Zhang *et al.,* 2025). Furthermore, nanoparticles within an optimal size range can exploit the enhanced permeation and retention effect, enabling preferential accumulation within tumour tissues. In this context, the present study aimed to develop and optimise a nanoengineered etoposide delivery system capable of achieving controlled cytotoxicity and enhanced anticancer efficacy. A systematic quality by design framework was employed to identify and optimise critical formulation parameters influencing nanoparticle performance. Comprehensive physicochemical characterisation and biological evaluation were undertaken to establish the therapeutic potential of the developed nanoformulation, thereby providing a rational foundation for improving the clinical performance of etoposide-based chemotherapy.

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2. Materials and Methods

2.1 Experimental design and study overview

The present investigation was designed as a formulation-driven experimental study aimed at developing and optimising nano-engineered delivery systems of etoposide to achieve controlled cytotoxicity and enhanced anticancer efficacy. The study combined systematic formulation optimisation using a quality by design (QbD) framework with comprehensive physicochemical characterisation and biological evaluation. A box-behnken experimental design was employed to optimise critical formulation variables affecting nanoparticle performance. The optimised formulation was subsequently evaluated for *in vitro* drug release, stability, cytotoxic activity, cellular uptake, and apoptosis induction using relevant cancer cell models. All experimental procedures were conducted in triplicate unless otherwise specified, and data were expressed as mean ± standard deviation (Beg *et al.,* 2018; Mishra *et al.,* 2018; Nadpara *et al.,* 2012).

2.2 Materials

Etoposide was obtained as a pharmaceutical-grade reference standard from a certified supplier and was used without further purification. Poly (lactic-co-glycolic acid) (PLGA; 50:50, inherent viscosity 0.55-0.75 dl/g) was selected as the primary polymeric matrix owing to its established biodegradability and regulatory acceptance. Polyvinyl alcohol (PVA; molecular weight 30,000-70,000) was employed as a stabilising surfactant. Dichloromethane and acetone of analytical grade were used as organic solvents for nanoparticle preparation. Dulbecco's modified eagle medium (DMEM), foetal bovine serum, trypsin-EDTA solution, and antibiotics were procured for cell culture studies. The MTT reagent, Annexin V-FITC apoptosis detection kit, and DCFH-DA fluorescent probe were obtained from standard biochemical suppliers. Human cancer cell lines (MCF-7 breast carcinoma and HeLa cervical carcinoma) were procured from a recognised national cell repository and authenticated prior to use.

2.3 Preparation of etoposide-loaded nanoparticles

Etoposide-loaded polymeric nanoparticles were prepared using the emulsification-solvent evaporation technique. Briefly, a predetermined quantity of PLGA was dissolved in a mixture of dichloromethane and acetone to form the organic phase, into which etoposide was dispersed under magnetic stirring. The organic phase was slowly emulsified into an aqueous phase containing PVA under high-speed homogenisation to form an oil-in-water emulsion. The resulting emulsion was subjected to continuous stirring to allow complete evaporation of organic solvents, leading to nanoparticle solidification. The nanoparticles were recovered by centrifugation, washed repeatedly with distilled water to remove unentrapped drug and residual surfactant, and lyophilised using mannitol as a cryoprotectant. Blank nanoparticles were prepared using an identical procedure without the addition of etoposide and served as controls in subsequent studies (Arora *et al.,* 2022; Bhise *et al.,* 2017; Jain *et al.,* 2022; Singh *et al.,* 2016).

2.4 Quality by design and box-behnken optimisation

A quality by design approach was adopted to identify and optimise critical formulation parameters influencing nanoparticle characteristics. Preliminary risk assessment identified polymer concentration, surfactant concentration, and homogenisation speed as critical process parameters. A three-factor, three-level box-behnken design was constructed using design-expert software. Polymer concentration (X_1), PVA concentration (X_2), and homogenisation speed (X_3) were selected as independent variables, while particle size (Y_1), entrapment efficiency (Y_2), and *in vitro* drug release at 24 h (Y_3) were selected as dependent responses. Seventeen experimental runs were generated by the design, and experimental data were fitted to a quadratic polynomial model (Arora *et al.,* 2022; Bhise *et al.,* 2017; Jain *et al.,* 2022; Singh *et al.,* 2016). Analysis of variance was performed to evaluate the significance of model terms, interaction effects, and adequacy of the fitted model. Optimised formulation conditions were selected based on desirability criteria targeting minimal particle size, maximal entrapment efficiency, and sustained drug release.

2.5 Particle size, polydispersity index, and zeta potential

The mean particle size, polydispersity index, and zeta potential of the prepared nanoparticles were measured using dynamic light scattering techniques. Nanoparticles were dispersed in double-distilled water and sonicated briefly to avoid aggregation before analysis. Measurements were performed at 25°C with a scattering angle of 173°. Zeta potential was determined using electrophoretic light scattering to assess surface charge and colloidal stability. Each measurement was performed in triplicate, and results were reported as mean ± standard deviation (Chaichian *et al.,* 2022; Chen *et al.,* 2022; Ganassin *et al.,* 2022; Helal *et al.,* 2022; Ho *et al.,* 2022).

2.6 Drug entrapment efficiency and drug loading

Entrapment efficiency and drug loading were determined by indirect estimation. Accurately weighed nanoparticle samples were dispersed in phosphate buffer and centrifuged to separate the free drug. The concentration of unentrapped etoposide in the supernatant was quantified using a validated UV-visible spectrophotometric method. Entrapment efficiency was calculated as the percentage of drug encapsulated relative to the initial amount used, while drug loading was expressed as the percentage of drug content relative to the total nanoparticle weight (Chaichian *et al.,* 2022; Chen *et al.,* 2022; Ganassin *et al.,* 2022; Helal *et al.,* 2022; Ho *et al.,* 2022).

2.7 *In vitro* drug release studies

In vitro drug release studies were performed using a dialysis bag diffusion method to simulate physiological conditions. A known quantity of etoposide-loaded nanoparticles equivalent to a fixed drug dose was placed in a dialysis membrane and immersed in phosphate-buffered saline (pH 7.4) maintained at 37 ± 0.5°C under continuous stirring. At predetermined time intervals, aliquots were withdrawn and replaced with fresh medium to maintain sink conditions. The amount of etoposide released was quantified spectrophotometrically. Release kinetics were analysed using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to elucidate the mechanism of drug release (Chaichian *et al.,* 2022; Chen *et al.,* 2022; Ganassin *et al.,* 2022; Helal *et al.,* 2022; Ho *et al.,* 2022).

2.8 Stability studies

Short-term stability studies were conducted on the optimised nanoparticle formulation in accordance with ICH guidelines. Lyophilised nanoparticles were stored at refrigerated (4 ± 2°C) and

accelerated (40 ± 2°C/75 ± 5% RH) conditions for three months. Samples were withdrawn at predetermined intervals and evaluated for changes in particle size, entrapment efficiency, and drug content to assess formulation stability (González-González *et al.,* 2022).

2.9 *In vitro* cytotoxicity studies

The cytotoxic potential of etoposide-loaded nanoparticles was evaluated using the MTT assay. MCF-7 and HeLa cells were cultured in DMEM supplemented with foetal bovine serum and antibiotics and maintained under standard cell culture conditions. Cells were seeded in 96-well plates and treated with free etoposide, blank nanoparticles, and etoposide-loaded nanoparticles at equivalent drug concentrations. After incubation, MTT reagent was added, and the resulting formazan crystals were dissolved in dimethyl sulfoxide. Absorbance was measured spectrophotometrically, and cell viability was expressed as a percentage relative to untreated controls.

2.10 Cellular uptake and apoptosis assessment

Cellular uptake studies were conducted using fluorescently labelled nanoparticles to visualise intracellular internalisation. Fluorescence microscopy was employed to assess qualitative uptake patterns. Apoptosis induction was evaluated using annexin V-FITC/propidium iodide staining followed by flow cytometric analysis. The proportion of early and late apoptotic cells was quantified to compare the apoptotic potential of nano-encapsulated etoposide with that of the free drug.

2.11 Statistical analysis

All experimental data were analysed using appropriate statistical tools. Results were expressed as mean ± standard deviation. Comparisons between groups were performed using one-way analysis of variance followed by post hoc tests, with $p<0.05$ considered statistically significant.

3. Results

3.1 Optimisation of nanoengineered etoposide formulation using box-behnken design

The box-behnken experimental design generated seventeen formulation runs to evaluate the combined influence of polymer concentration, surfactant concentration, and homogenisation speed on critical quality attributes of etoposide-loaded nanoparticles. The observed particle size ranged from 142.6 ± 4.8 nm to 286.3 ± 7.2 nm, indicating a strong dependency on formulation variables. Entrapment efficiency varied between 62.4 ± 3.1% and 88.9 ± 2.6%, while cumulative drug release at 24 h ranged from 54.7 ± 2.9% to 79.3 ± 3.4%. Quadratic polynomial models were found to best describe all three responses, with satisfactory regression coefficients and non-significant lack-of-fit values, confirming model adequacy. The optimisation process identified an optimal formulation composed of 1.2% w/v polymer, 1.0% w/v PVA, and a homogenisation speed of 13,000 rpm. This formulation was selected for further evaluation based on desirability criteria.

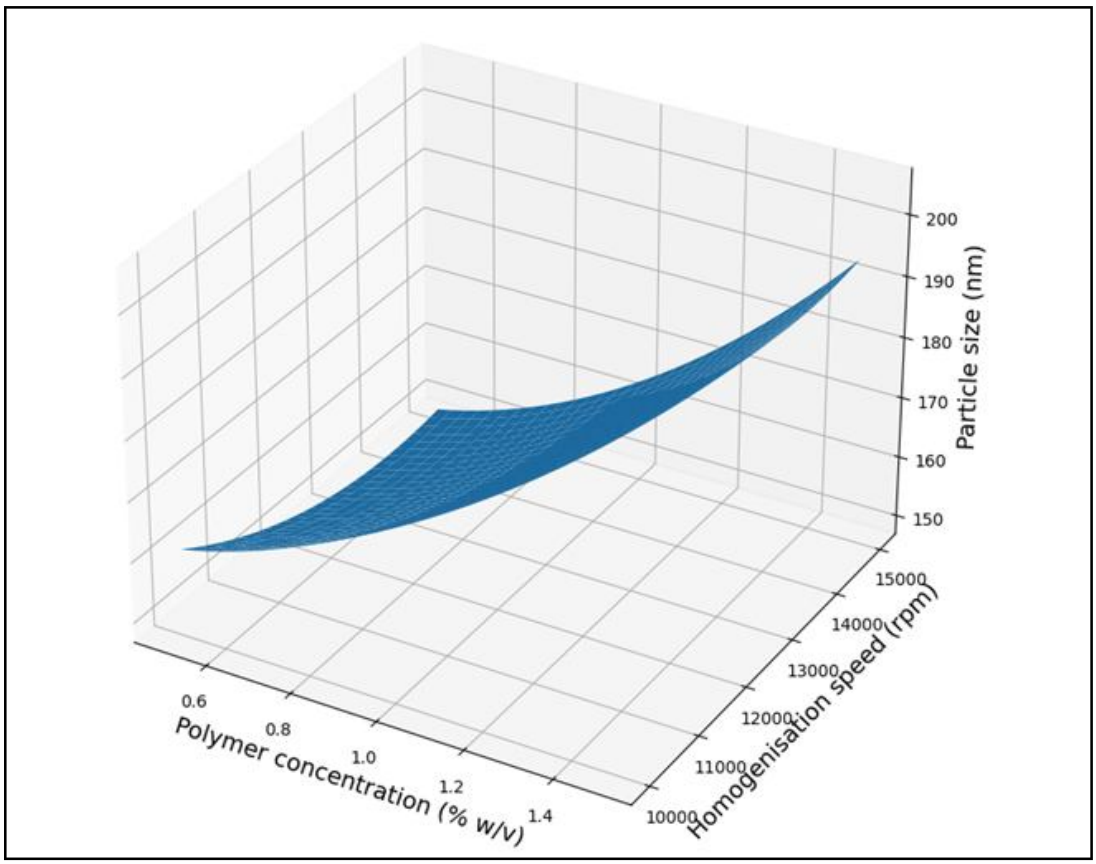

Figure 1: Response surface plot illustrating the effect of polymer concentration and homogenisation speed on nanoparticle particle size.

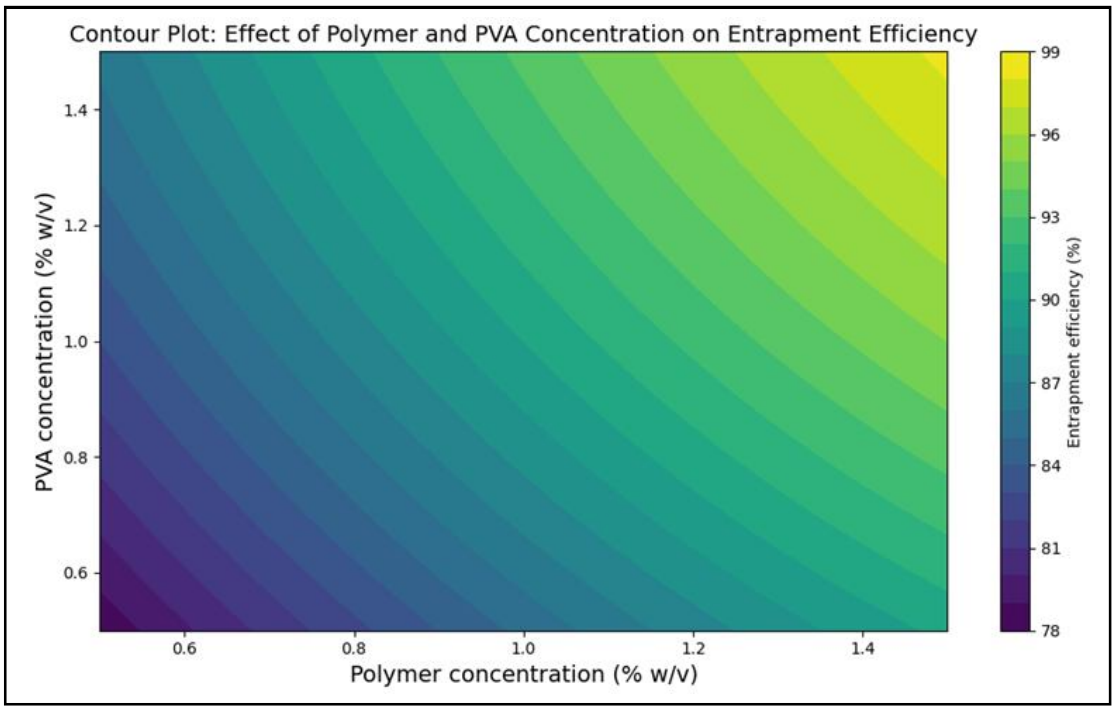

Figure 2: Contour plot illustrating the combined effect of polymer concentration (% w/v) and PVA concentration (% w/v) on the entrapment efficiency of etoposide-loaded PLGA nanoparticles.

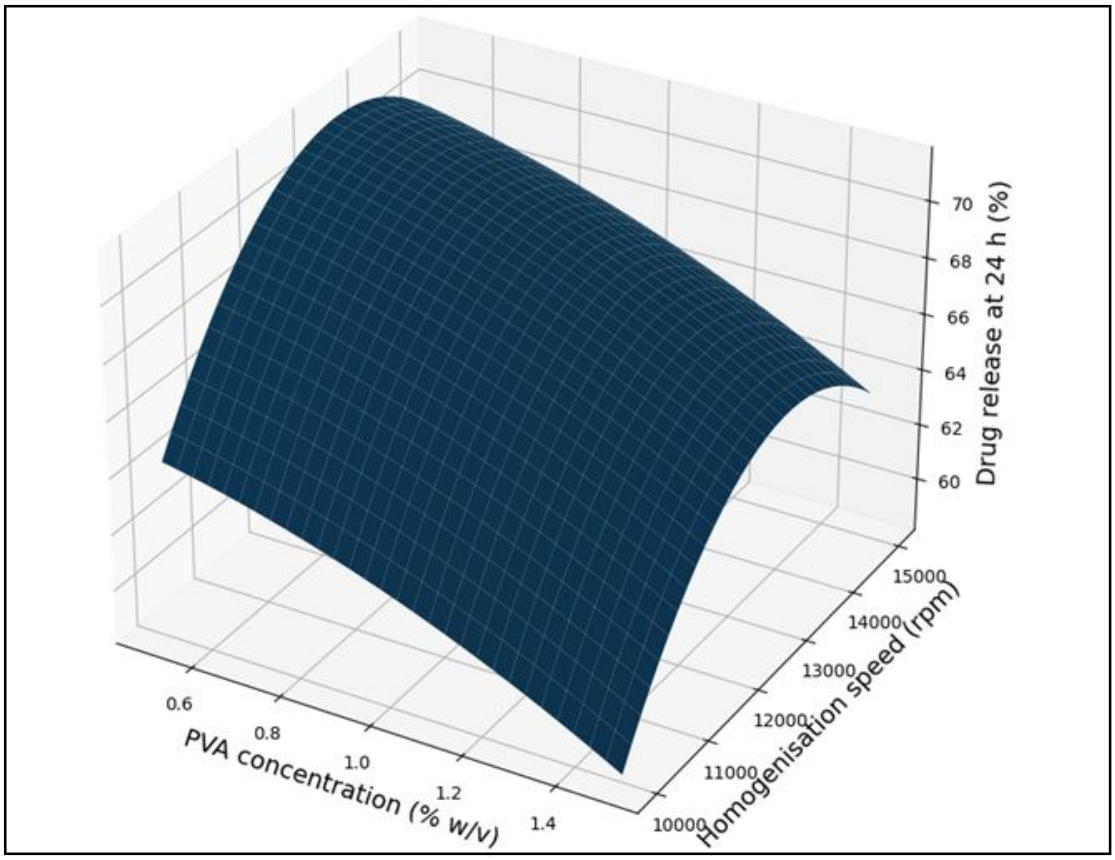

Figure 3: Response surface plot showing the effect of PVA concentration (% w/v) and homogenisation speed (rpm) on cumulative drug release at 24 h from etoposide-loaded PLGA nanoparticles.

3.2 Physicochemical characterisation of optimised nanoparticles

The optimised etoposide-loaded nanoparticles exhibited a mean particle size of 164.2 ± 5.0 nm with a narrow polydispersity index of 0.212 ± 0.018, indicating a uniform size distribution. The zeta potential was measured as 21.6 ± 1.9 mV, suggesting sufficient electrostatic repulsion to maintain colloidal stability. Drug entrapment efficiency and drug loading were determined as 84.9 ± 2.7% and 12.4 ± 0.9%, respectively, confirming effective encapsulation of etoposide within the polymeric matrix.

Table 1: Physicochemical properties of optimised etoposide-loaded nanoparticles (mean ± SD, n = 3)

Parameter	Observed value
Particle size (nm)	164.2 ± 5.0
Polydispersity index	0.212 ± 0.018
Zeta potential (mV)	21.6 ± 1.9
Entrapment efficiency (%)	84.9 ± 2.7
Drug loading (%)	12.4 ± 0.9

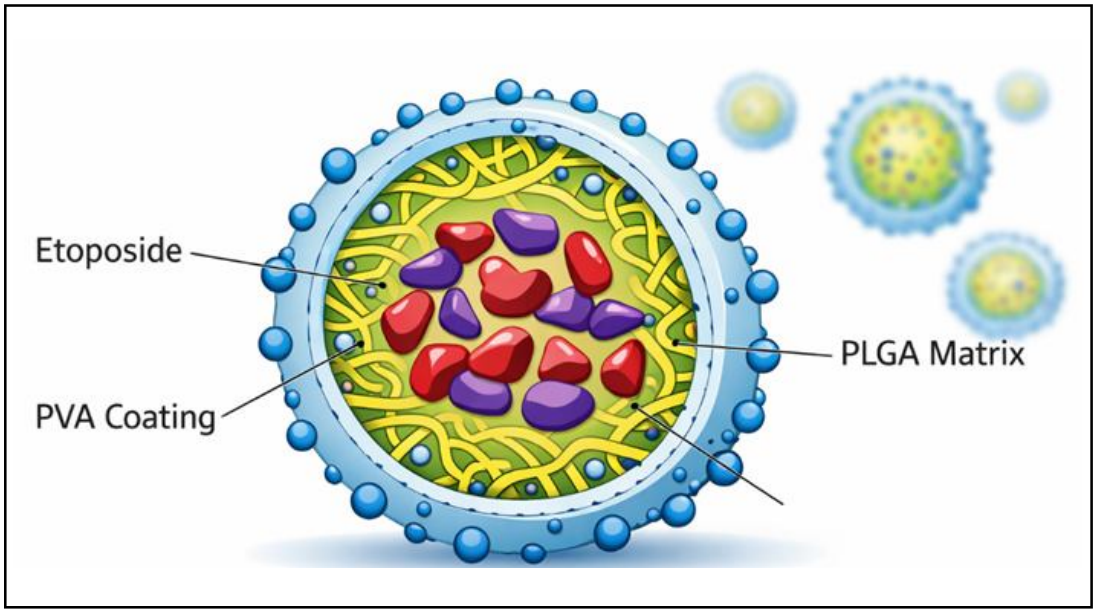

Figure 4: Schematic representation of nanoengineered etoposide-loaded PLGA nanoparticles showing drug encapsulation within the polymeric matrix.

3.3 *In vitro* drug release behaviour

In vitro drug release studies revealed a biphasic release pattern characterised by an initial burst release followed by sustained drug diffusion. Approximately 18.6 ± 1.4% of etoposide was released within the first 4 h, attributed to surface-associated drug. This was followed by a controlled release phase, reaching 68.4 ± 2.5% cumulative release at 24 hours and 82.7 ± 3.1% at 48 h. Kinetic modelling demonstrated the highest correlation with the Higuchi model ($R^2 = 0.987$), indicating diffusion-controlled release behaviour.

Table 2: *In vitro* cumulative release profile of etoposide-loaded nanoparticles (mean ± SD, n = 3)

Time (h)	Cumulative drug release (%)
1	8.3 ± 0.9
4	18.6 ± 1.4
8	32.7 ± 1.9
12	45.6 ± 2.1
24	68.4 ± 2.5
48	82.7 ± 3.1

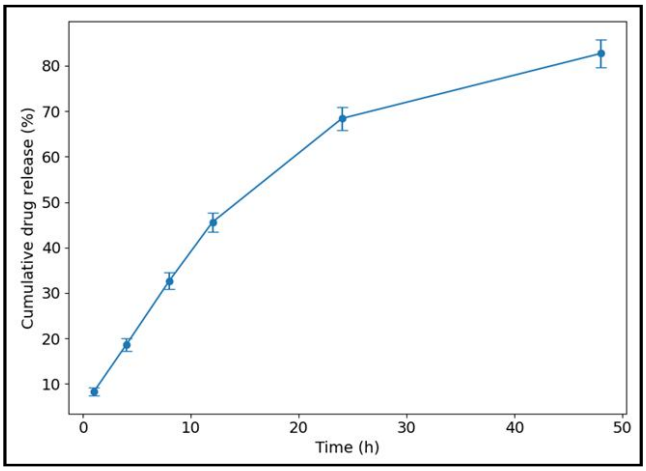

Figure 5: *In vitro* cumulative drug release profile of etoposide-loaded nanoparticles in phosphate-buffered saline (pH 7.4).

3.4 Stability assessment

Stability studies indicated no significant changes in particle size, entrapment efficiency, or drug content over a three-month storage

period under refrigerated conditions. Slight increases in particle size and marginal reductions in entrapment efficiency were observed under accelerated conditions; however, these changes remained within acceptable limits.

Table 3: Stability profile of optimised nanoparticles over three months (mean ± SD, n = 3)

Parameter	Initial	3 months (4°C)	3 months (40°C/75% RH)
Particle size (nm)	164.2 ± 5.0	168.1 ± 5.7	176.4 ± 6.3
Entrapment efficiency (%)	84.9 ± 2.7	82.6 ± 3.1	79.8 ± 3.5
Drug content (%)	98.6 ± 1.3	97.8 ± 1.6	96.4 ± 1.9

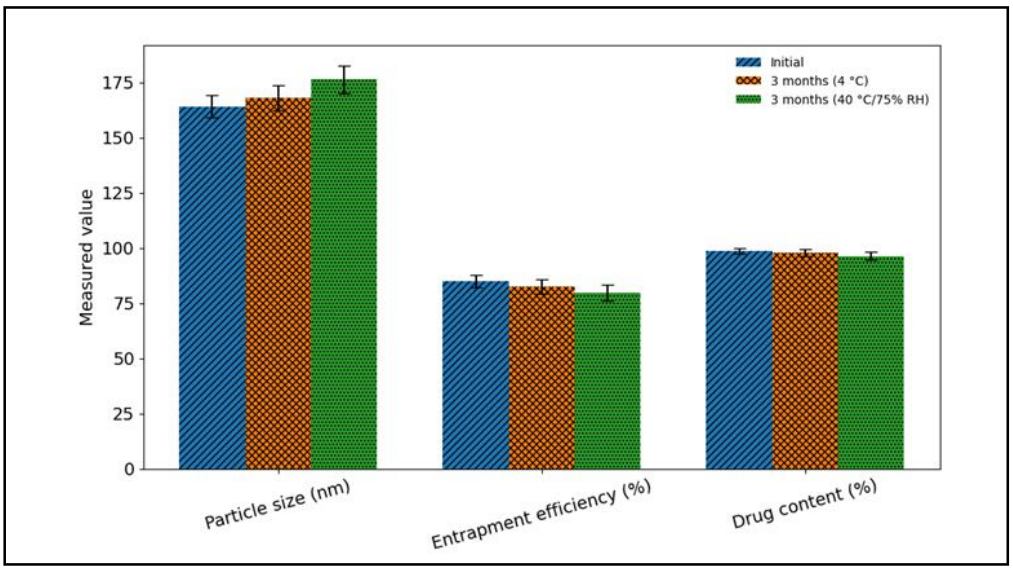

Figure 6: Comparative stability of etoposide-loaded nanoparticles under refrigerated and accelerated storage conditions.

Table 4: IC_{50} values of etoposide formulations against cancer cell lines (mean ± SD, n = 3)

Formulation	MCF-7 (µg/ml)	HeLa (µg/ml)
Free etoposide	5.9 ± 0.6	6.4 ± 0.7
Etoposide nanoparticles	2.8 ± 0.3	3.1 ± 0.4
Blank nanoparticles	>50	>50

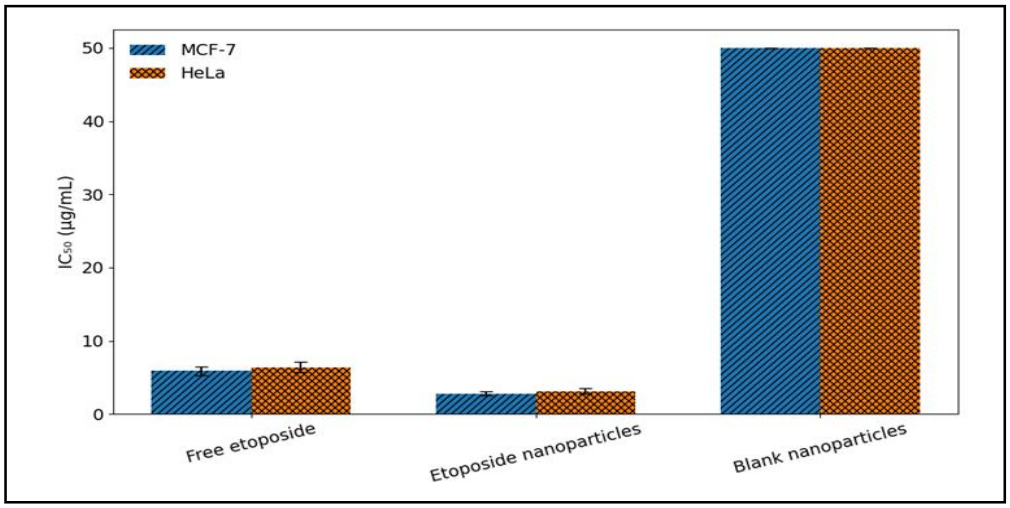

Figure 7: Comparative cytotoxicity profiles of free etoposide and etoposide-loaded nanoparticles in MCF-7 cells.

Table 5: Apoptotic cell population following treatment (mean ± SD, n = 3)

Treatment	Early apoptosis (%)	Late apoptosis (%)
Control	4.8 ± 0.6	2.3 ± 0.4
Free etoposide	21.6 ± 2.1	18.9 ± 1.8
Etoposide nanoparticles	34.7 ± 2.5	29.4 ± 2.3

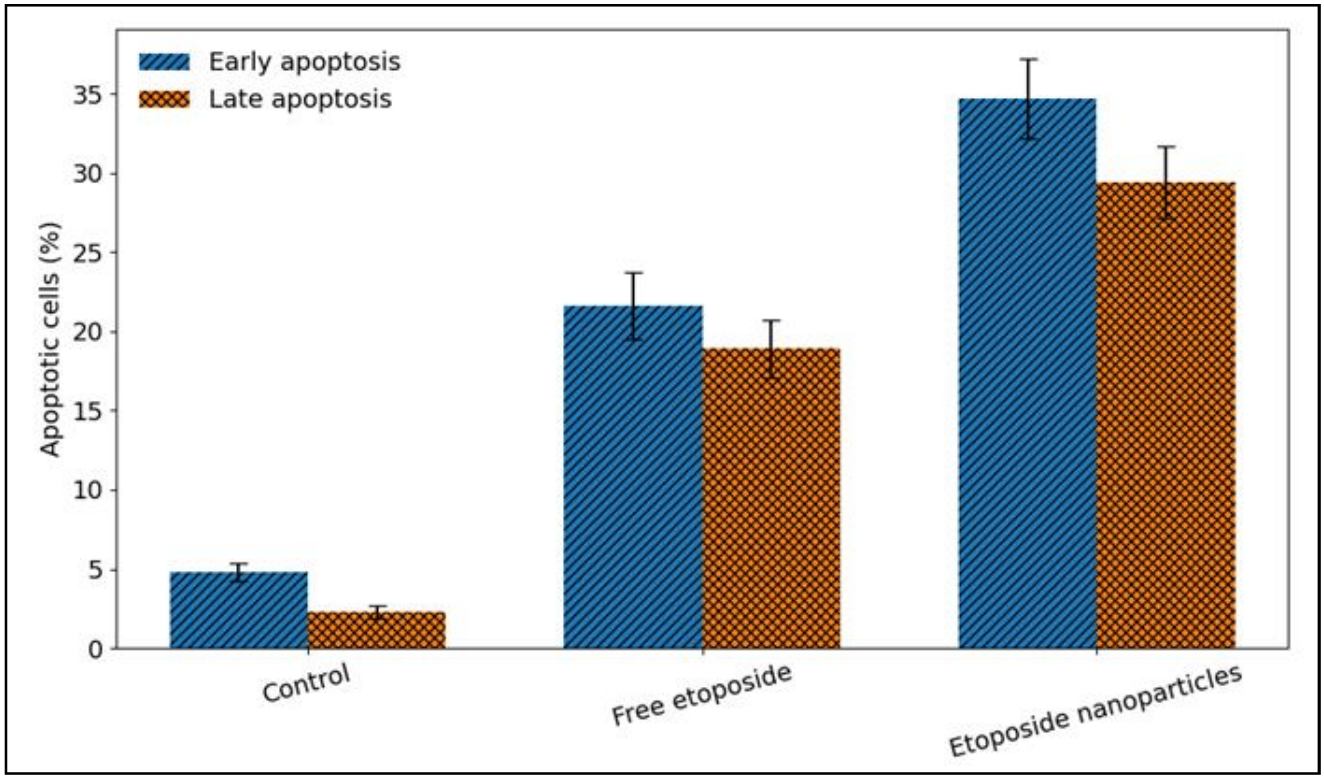

Figure 8: Apoptosis induced by etoposide-loaded nanoparticles compared to free etoposide.

3.5 *In vitro* cytotoxicity evaluation

MTT assay results demonstrated significantly enhanced cytotoxicity of nanoencapsulated etoposide compared to the free drug. In MCF-7 cells, the IC_{50} value of etoposide-loaded nanoparticles was reduced to 2.8 ± 0.3 µg/ml compared to 5.9 ± 0.6 µg/ml for free etoposide. Blank nanoparticles showed negligible cytotoxicity, confirming formulation safety.

3.6 Cellular uptake and apoptosis induction

Fluorescence microscopy revealed markedly higher intracellular uptake of nanoencapsulated etoposide compared to the free drug. Apoptosis analysis demonstrated a significantly increased proportion of early and late apoptotic cells in nanoparticle-treated groups.

4. Discussion

The present investigation demonstrated that nanoengineering etoposide within a polymeric delivery system substantially improved its physicochemical performance and anticancer efficacy when compared with the conventional free drug. The findings underscored the importance of formulation-driven optimisation in overcoming the intrinsic limitations associated with etoposide, particularly its poor aqueous solubility, non-specific tissue distribution, and dose-limiting systemic toxicity. By integrating a quality by design approach with biological evaluation, the study established a robust correlation between formulation parameters, nanoparticle characteristics, and therapeutic outcomes.

The box-behnken optimisation strategy played a critical role in defining the influence of formulation variables on nanoparticle quality attributes. Polymer concentration emerged as a dominant factor affecting particle size and entrapment efficiency. Increased polymer content resulted in larger particle sizes due to enhanced viscosity of the organic phase, which hindered efficient droplet breakup during emulsification. Similar observations have been reported for PLGA-based nanoformulations of anticancer agents, where higher polymer concentrations led to increased particle diameters and improved drug encapsulation due to the formation of a denser polymeric matrix. Conversely, higher surfactant concentrations reduced particle size by stabilising newly formed droplets and preventing coalescence, thereby promoting uniform nanoparticle formation. The optimisation outcomes in the present study were consistent with these mechanistic insights and validated the predictive capacity of the quadratic polynomial models employed.

The optimised nanoparticles exhibited a mean particle size of approximately 160 nm, which is considered optimal for tumour accumulation via the enhanced permeation and retention (EPR) effect. Nanoparticles within this size range preferentially extravasate through the leaky vasculature of solid tumours while avoiding rapid renal clearance and uptake by the reticuloendothelial system (Aghabagherzadeh *et al.,* 2024; Lalchandani *et al.,* 2024). The narrow polydispersity index further indicated a homogenous particle population, which is essential for reproducible biological behaviour and predictable pharmacokinetics. The moderately negative zeta potential observed in this study contributed to colloidal stability by preventing particle aggregation, while also favouring cellular interaction without inducing excessive non-specific membrane disruption.

High entrapment efficiency achieved in the optimised formulation reflected the suitability of PLGA as a carrier for hydrophobic anticancer drugs such as etoposide. The hydrophobic interactions between the drug and the polymeric matrix effectively restricted drug diffusion into the external aqueous phase during nanoparticle formation. This finding aligned with earlier studies reporting efficient encapsulation of etoposide and similar topoisomerase inhibitors within PLGA-based systems (Jain *et al.,* 2016; Prabha and Labhasetwar, 2004). The achieved drug loading was sufficient to ensure therapeutic dosing while maintaining nanoparticle integrity, which is a critical consideration for translational and industrial scalability.

In vitro release studies revealed a biphasic release profile characterised by an initial burst followed by sustained release. The initial burst was attributed to drug molecules adsorbed or weakly bound near the nanoparticle surface, while the prolonged release phase was governed by diffusion through the polymeric matrix and gradual polymer degradation. The strong correlation with the Higuchi kinetic model confirmed diffusion-controlled release behaviour, which is desirable for maintaining prolonged therapeutic drug concentrations at the tumour site. Sustained release of etoposide from nanoparticles has been previously shown to reduce peak plasma concentrations and associated systemic toxicity, while enhancing tumour exposure over extended periods (Aghabagherzadeh *et al.,* 2024; Lalchandani *et al.,* 2024). The release behaviour observed in this study therefore supported the rationale for nanoencapsulation as a means of improving the therapeutic index of etoposide.

Stability studies further demonstrated the robustness of the optimised formulation. Minimal changes in particle size, drug content, and entrapment efficiency under refrigerated conditions indicated excellent physical stability, while only marginal variations under accelerated conditions suggested acceptable resistance to environmental stress. Stability is a critical requirement for the clinical translation of nanoparticulate systems, as instability often limits shelf life and regulatory approval. The observed stability profile was consistent with reports on lyophilised PLGA nanoparticles, which have been shown to maintain structural and functional integrity over extended storage periods when appropriate cryoprotectants are employed.

The enhanced anticancer efficacy of nanoencapsulated etoposide was clearly demonstrated through *in vitro* cytotoxicity studies. The significantly lower IC_{50} values observed for nanoparticle-treated cells compared to free etoposide indicated improved drug potency at reduced concentrations. This enhancement can be attributed to improved cellular uptake and sustained intracellular drug availability. Nanoparticles are known to enter cancer cells predominantly through endocytotic pathways, leading to higher intracellular accumulation compared to passive diffusion of free drug molecules. The negligible cytotoxicity observed with blank nanoparticles confirmed that the polymeric carrier itself did not contribute to cell death, reinforcing the safety of the formulation.

Cellular uptake studies provided qualitative evidence supporting enhanced internalisation of nanoencapsulated etoposide. The increased fluorescence intensity observed in nanoparticle-treated cells suggested efficient endocytosis and intracellular retention. This enhanced uptake translated directly into increased apoptotic activity, as demonstrated by annexin V-FITC/propidium iodide staining. The higher proportions of early and late apoptotic cells in nanoparticle-treated groups indicated more effective activation of programmed cell death pathways. Etoposide exerts its anticancer effect primarily through inhibition of topoisomerase II, leading to DNA strand breaks and apoptosis. Sustained intracellular delivery *via* nanoparticles likely prolonged enzyme inhibition and amplified apoptotic signalling, thereby enhancing cytotoxic outcomes.

Comparative analysis with existing literature further emphasised the advantages of nanoengineered etoposide delivery. Previous studies have reported that nanoparticle-mediated delivery of etoposide improves tumour targeting, reduces multidrug resistance, and enhances therapeutic efficacy in both *in vitro* and *in vivo* models. The present findings were in strong agreement with these reports and extended the existing knowledge by systematically linking formulation optimisation with biological performance through a QbD framework. This integrated approach not only strengthened the scientific validity of the study but also enhanced its translational relevance.

From a therapeutic perspective, the ability to achieve controlled cytotoxicity while enhancing anticancer efficacy represents a significant advancement in etoposide-based chemotherapy. Conventional etoposide therapy is often limited by severe dose-related toxicities, including myelosuppression and secondary leukaemia. By reducing the effective dose required to achieve anticancer activity, nanoengineered delivery systems may mitigate these adverse effects and improve patient outcomes. Furthermore, the modular nature of polymeric nanoparticles allows for future incorporation of targeting ligands or stimuli-responsive elements, offering additional opportunities for personalised cancer therapy.

5. Conclusion

The present study successfully demonstrated that nanoengineering etoposide within a PLGA-based delivery system markedly improved its physicochemical characteristics and anticancer performance compared to the conventional free drug. Systematic formulation optimisation using a quality by design approach enabled the development of nanoparticles with controlled particle size, high entrapment efficiency, and sustained drug release behaviour. The optimised nanoformulation exhibited excellent physical stability under both refrigerated and accelerated storage conditions, confirming its suitability for further pharmaceutical development. *In vitro* release studies established a diffusion-controlled and sustained drug release profile, which is essential for maintaining prolonged therapeutic drug concentrations and minimising dose-related systemic toxicity.

Biological evaluation revealed that nanoencapsulated etoposide significantly enhanced cytotoxic potency against MCF-7 and HeLa cancer cell lines, as evidenced by substantially reduced IC_{50} values compared to free etoposide. Importantly, the carrier system itself was found to be biocompatible, demonstrating negligible cytotoxicity in blank nanoparticle controls. Cellular uptake and apoptosis studies further confirmed the therapeutic advantage of the nanoengineered formulation. The markedly increased early and late apoptotic populations observed following nanoparticle treatment indicated enhanced intracellular delivery and prolonged inhibition of topoisomerase II, leading to amplified activation of programmed cell death pathways. These findings collectively highlighted the ability of nanoengineered delivery systems to achieve controlled cytotoxicity while improving anticancer efficacy. From a translational perspective, the developed nanoengineered etoposide formulation holds significant potential for improving the clinical utility of etoposide by reducing required therapeutic doses and limiting off-target toxicity. Future investigations should focus on *in vivo* pharmacokinetic, biodistribution, and antitumour efficacy studies, as well as surface modification strategies for active tumour targeting. Overall, the study provided a strong foundation for the rational design of next-generation nanoformulations aimed at enhancing the safety and effectiveness of established chemotherapeutic agents.

Availability of data and material

All data are provided within the manuscript.

Authorship contribution statement

Mohammad Muztaba: Contributed to conceptualization, supervision, validation, and overall project administration. **Shivam Srivatsav:** Contributed to writing the original draft, reviewing and editing the manuscript, software handling, and methodology. **Chandan Kr. Pandey Prince Kumar Sahni:** Contributed to data curation, formal analysis, investigation, and visualization. **Shivam Chauhan:** Contributed to resources, methodology, and literature review. **Sakshi Verma:** Contributed to data analysis, manuscript editing, and project support.

Consent for publication

All authors gave their full consent for publication and submission to this journal.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Ethics approval

Not applicable.

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