Design, Optimization, and In Vitro Evaluation of Doxorubicin-Loaded PLGA Nanoparticles for Targeted Cancer Therapy

[1*] Dr. Vishal S. More, [2] Madhuri B. Kanawade, [3] Pratiksha S. Bidwai, [4] Shatabdi Ghose, [5] Aminur Rahman, [6] Sejal D. Jadhav

[1*]Professor and Head, Department of Pharmaceutical Chemistry, Amrutvahini College of Pharmacy, Sangamner, Ahmednagar, 422608, Maharashtra, India. E-mail-Id: nirmalpharmasolutions@gmail.com

[2]Assistant Professor, Department of Pharmaceutical Chemistry, Amrutvahini College of Pharmacy, Sangamner, Ahmednagar, 422608, Maharashtra, India.

[3]Department of Pharmaceutical Quality Assurance, Amrutvahini College of Pharmacy, Sangamner, Ahmednagar, 422608, Maharashtra, India.

[4]Assistant Professor, Department of Pharmacology, Girijananda Chowdhury University, Azara, Hathkhowapara, Guwahati, 781017, Assam, India.

[5]Department of Pharmacology, Girijananda Chowdhury University, Azara, Hathkhowapara, Guwahati, 781017, Assam, India.

[6]Navsahyadri Institute of Pharmacy, Naigoan, Nasarapur, Pune, 412213, Maharashtra, India.

Address for Correspondence:

*Dr. Vishal S. More**

Professor and Head, Department of Pharmaceutical Chemistry, Amrutvahini College of Pharmacy, Sangamner, Ahmednagar, 422608, Maharashtra, India.

E-mail-Id: nirmalpharmasolutions@gmail.com

ABSTRACT:

Cancer remains a major global health challenge, necessitating the development of effective and safe therapeutic strategies. The present study aimed to design and evaluate doxorubicin hydrochloride (DOX)-loaded poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles for targeted cancer therapy. Nanoparticles were prepared using the oil-in-water emulsion–solvent evaporation method and optimized by varying the drug-to-polymer ratio. The prepared formulations were characterized in terms of particle size, polydispersity index (PDI), entrapment efficiency (EE%), drug loading (DL%), in vitro drug release, and release kinetics.

The optimized formulation (F4) exhibited a mean particle size of 132 ± 3 nm with a low PDI (0.19 ± 0.01), indicating a uniform size distribution suitable for tumor targeting via the enhanced permeability and retention effect. The entrapment efficiency and drug loading were 86.3 ± 1.4% and 9.5 ± 0.4%, respectively, demonstrating efficient drug incorporation within the PLGA matrix. In vitro release studies revealed a biphasic pattern with an initial mild burst release, followed by sustained drug release up to 72 h, achieving 85.2 ± 2.3% cumulative release. Release kinetic modelling indicated that drug release followed the Higuchi model (R^2 = 0.978), suggesting a diffusion-controlled mechanism with Fickian diffusion.

Overall, the developed DOX-loaded PLGA nanoparticles demonstrated favourable physicochemical properties and controlled release characteristics, indicating their potential as a promising nanotechnology-based approach for improving therapeutic efficacy and reducing systemic toxicity in targeted cancer therapies.

KEYWORDS: Doxorubicin hydrochloride, PLGA nanoparticles, Targeted drug delivery, Controlled release, Cancer therapy

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1. INTRODUCTION:

Cancer remains one of the leading causes of morbidity and mortality worldwide and represents a major global public health challenge. According to the World Health Organization (WHO), cancer accounted for approximately 10 million deaths in 2020, making it one of the most significant causes of mortality globally[1] Recent global cancer statistics (GLOBOCAN 2022) estimate that over 19–20 million new cancer cases were diagnosed worldwide in 2022, and this number is projected to rise to nearly 28–30 million cases annually by 2040 due to population growth, aging, and lifestyle-related risk factors[2]. The increasing incidence and economic burden of cancer

underscore the urgent need for more effective and safer therapeutic strategies against it.

Despite substantial advancements in diagnostic and therapeutic modalities, chemotherapy remains a fundamental component of cancer treatment strategies. Among the widely used chemotherapeutic agents, doxorubicin hydrochloride (DOX), an anthracycline antibiotic, has been extensively employed in the treatment of various solid tumors and hematological malignancies owing to its broad-spectrum anticancer activity. However, conventional chemotherapy, including DOX therapy, is associated with considerable limitations that compromise therapeutic outcomes and patient's quality of life.

1.1 Limitations of Conventional Chemotherapy:

Conventional chemotherapeutic agents exert cytotoxic effects by targeting rapidly dividing cells; however, they lack specificity for malignant cells. Consequently, healthy proliferating tissues such as bone marrow, gastrointestinal epithelium, and hair follicles are also adversely affected[3]. This non-selective mechanism results in severe systemic toxicities, including myelosuppression, mucositis, nausea, vomiting, alopecia, and immunosuppression, often necessitating dose reduction or treatment discontinuation[4].

In the case of Doxorubicin hydrochloride (DOX), dose-dependent cardiotoxicity remains one of the most serious adverse effects, significantly limiting its cumulative lifetime dose[5]. Furthermore, many anticancer drugs, including DOX, may exhibit suboptimal tumor accumulation due to rapid systemic clearance and unfavourable pharmacokinetic profiles[6]. The development of multidrug resistance (MDR) presents a major clinical challenge. Overexpression of efflux transporters, such as P-glycoprotein (P-gp), reduces intracellular drug concentrations, thereby diminishing therapeutic responses and contributing to tumor recurrence.

Additionally, biological barriers such as the dense extracellular matrix of tumors, abnormal tumor vasculature, and the blood–brain barrier further limit effective drug penetration into tumor tissues[7]. Collectively, these challenges highlight the need for innovative drug delivery systems capable of improving tumor selectivity while minimizing systemic toxicity, particularly for potent agents such as DOX.

1.2 Need for Targeted Drug Delivery:

Targeted drug delivery systems aim to selectively direct therapeutic agents to tumor tissues while minimizing exposure to normal cells. This strategy enhances the therapeutic index and reduces dose-limiting toxicities[8]. Tumor tissues possess unique pathophysiological characteristics that can be exploited for selective drug accumulation, including enhanced vascular permeability, impaired lymphatic drainage, an acidic microenvironment, and overexpression of specific receptors[9].

Passive targeting is primarily based on the Enhanced Permeability and Retention (EPR) effect, which allows nanoscale carriers to preferentially accumulate in tumor tissues owing to leaky vasculature[10]. Active targeting further improves specificity by functionalizing drug carriers with ligands, antibodies, peptides, or small molecules that selectively bind to overexpressed tumor-associated receptors such as folate receptors, HER2, epidermal growth factor receptor (EGFR), and transferrin receptors[11].

For chemotherapeutic agents such as doxorubicin hydrochloride (DOX), targeted delivery strategies can enhance intracellular uptake, overcome multidrug resistance mechanisms, and reduce systemic toxicity, thereby significantly improving therapeutic outcomes[12].

1.3 Advantages of Nanoparticles in Cancer Therapy:

Nanotechnology has emerged as a transformative approach in oncology because of the unique physicochemical properties of nanoparticles, which are typically 10–200 nm in size[13]. Their nanoscale dimensions allow efficient tumor penetration and cellular internalization, while their large surface area-to-volume ratio enables high drug loading capacity and surface functionalization[14].

Nanoparticles offer multiple advantages for cancer therapy.

- Improved solubility and stability: Encapsulation enhances the solubility of hydrophobic drugs, including DOX, and protects them from premature degradation[15].
- Enhanced bioavailability: Nanocarriers prolong systemic circulation and reduce rapid renal clearance[16].
- Controlled and sustained release: Polymeric and lipid-based nanoparticles allow programmed drug release, thereby reducing the dosing frequency[17].
- Reduced systemic toxicity: Targeted accumulation minimizes damage to healthy tissues and may reduce doxorubicin-associated cardiotoxicity.
- Multifunctionality: Nanoparticles can combine therapeutic and diagnostic agents (theranostics), enabling simultaneous

treatment and real-time monitoring of the therapeutic effect.

Several nanoparticle-based formulations, including liposomal doxorubicin and albumin-bound paclitaxel, have demonstrated clinical success, validating the translational potential of nanomedicine. Nanoparticle-based targeted drug delivery platforms continue to evolve as promising tools in precision oncology, particularly for optimizing the therapeutic performance of established anticancer agents, such as doxorubicin hydrochloride (DOX).

2. OBJECTIVE OF THE STUDY:

This study aimed to develop and evaluate a nanoparticle-based drug delivery system for targeted cancer therapy using doxorubicin hydrochloride (DOX) as the model anticancer drug.

The objectives included the formulation of DOX-loaded biodegradable nanoparticles, optimization of formulation parameters, physicochemical characterization, evaluation of drug loading and release behaviour, and assessment of in vitro anticancer activity.

The ultimate goal is to enhance the tumor-specific delivery of DOX, improve therapeutic efficacy, and reduce systemic toxicity using a nanotechnology-based approach.

3. MATERIALS AND METHODS:

3.1 Materials:

Doxorubicin hydrochloride (DOX) was selected as the model anticancer drug because of its broad-spectrum cytotoxic activity and extensive clinical application in solid tumor management[18]. Poly(D,L-lactide-co-glycolide) (PLGA; 50:50 lactide:glycolide ratio) was used as the biodegradable polymer matrix because of its excellent biocompatibility, controlled degradation kinetics, and approval for pharmaceutical applications[19,20].

Polyvinyl alcohol (PVA) was employed as a stabilizing surfactant to ensure colloidal stability and prevent nanoparticle aggregation during the formulation[21]. Dichloromethane (DCM) was used as the organic solvent to dissolve PLGA. All reagents were of analytical grade and used without further purification. Deionized water was used throughout the experimental procedure.

3.2 Method of Preparation:

3.2.1 Preparation of PLGA Nanoparticles by Emulsion–Solvent Evaporation Method:

Polymeric nanoparticles were prepared using the oil-in-water (O/W) emulsion-solvent evaporation technique, a well-established and widely reported method for the fabrication of biodegradable polymeric nanoparticles[20,14].

Five formulations (F1–F5) were prepared by varying the polymer concentration and drug-to-polymer ratio to optimize the nanoparticle characteristics.

Briefly, a predetermined quantity of PLGA was dissolved in dichloromethane to form an organic phase. Doxorubicin was dissolved in a minimal volume of distilled water and incorporated into the organic polymer solution according to the drug-to-polymer ratio of each formulation.

The organic phase was added dropwise to an aqueous phase containing 1–2% (w/v) polyvinyl alcohol under continuous magnetic stirring to form a primary emulsion. The resulting emulsion was subjected to probe sonication for 2-3 minutes at a controlled amplitude to reduce the droplet size and obtain a stable nanoemulsion.

The emulsion was stirred at room temperature for 3-4 hours to allow complete evaporation of the organic solvent, resulting in the formation of solidified nanoparticles.

The nanoparticle suspension was collected by centrifugation at 15,000 rpm for 20 min, washed three times with distilled water to remove unencapsulated drug and excess surfactant, and finally lyophilized to obtain a dry nanoparticle powder.

The prepared formulations (F1–F5) were stored at 4°C until further physicochemical and biological evaluation.

4. EVALUATION OF DOX-LOADED PLGA NANOPARTICLES:

The prepared formulations (F1–F5) were subjected to comprehensive physicochemical and in vitro evaluations to assess their suitability for targeted cancer-drug delivery. The evaluation parameters included particle size, polydispersity index (PDI), entrapment efficiency, drug loading, in-vitro drug release behaviour, release kinetic modelling, and cytotoxicity assessment. These studies were performed to determine nanoparticle stability, drug incorporation efficiency, release characteristics, and expected anticancer potential[7,12].

4.1 Particle Size and Polydispersity Index (PDI):

The mean particle size and polydispersity index (PDI) of the prepared formulations (F1–F5) were determined using Dynamic Light Scattering (DLS) at 25°C after appropriate dilution with deionized water[22].

Particle size analysis provides information on the hydrodynamic diameter of nanoparticles, whereas the PDI indicates the uniformity of the particle size distribution within the formulation[22,23]. Nanoparticles

in the range of 100–200 nm is considered optimal for tumor targeting via the Enhanced Permeability and Retention (EPR) effect[9,8]. A PDI value below 0.3 is generally accepted as indicative of uniform and monodispersed nanoparticle systems[23].

Table 1: Particle Size and Polydispersity Index of DOX-Loaded PLGA Nanoparticles

Formulation Code	Particle Size (nm)	PDI	Interpretation
F1	192 ± 5	0.34 ± 0.02	Slightly broad distribution
F2	168 ± 4	0.28 ± 0.01	Acceptable uniformity
F3	149 ± 3	0.22 ± 0.01	Uniform dispersion
F4	132 ± 3	0.19 ± 0.01	Narrow distribution
F5	158 ± 4	0.25 ± 0.02	Good uniformity

Interpretation:

Among the prepared formulations, F4 exhibited the smallest particle size (132 nm) and a low PDI value (0.19), indicating a highly uniform and monodispersed nanoparticle population. All optimized formulations demonstrated particle sizes within the ideal nanometric range (100–200 nm), which is suitable for enhanced tumor targeting and improved cellular uptake.

4.2 Entrapment Efficiency (EE%) and Drug Loading (DL%):

The entrapment efficiency and drug loading of DOX-loaded PLGA nanoparticles were determined using an indirect method. The nanoparticle suspension was centrifuged at 15,000 rpm for 20 min to separate the free (unencapsulated) drug from the nanoparticle pellet. The supernatant containing free DOX was collected and analyzed using UV–Visible spectrophotometry at $\lambda max \approx 480$ nm[19,20,17].

The amount of encapsulated drug was calculated by subtracting the free drug from the total amount of drug added during the formulation.

Formulas Used:

$$EE\ (\%) = [(\text{Total Drug} - \text{Free Drug}) / \text{Total Drug}] \times 100$$

$$DL\ (\%) = (\text{Entrapped Drug} / \text{Total Nanoparticle Weight}) \times 100$$

Where:

- Total Drug = Amount of DOX initially added
- Free Drug = Amount of DOX detected in supernatant
- Entrapped Drug = Total Drug − Free Drug

Table 2: Entrapment Efficiency and Drug Loading of DOX-Loaded PLGA Nanoparticles

Formulation Code	**EE (%)**	**DL (%)**	**Interpretation**
F1	68.5 ± 2.3	6.1 ± 0.4	Moderate encapsulation
F2	74.8 ± 1.9	7.4 ± 0.3	Improved loading
F3	81.6 ± 1.6	8.7 ± 0.5	High encapsulation
F4	86.3 ± 1.4	9.5 ± 0.4	Very high encapsulation
F5	78.2 ± 2.1	8.1 ± 0.3	Good loading

Interpretation:

The entrapment efficiency of the prepared formulations ranged from 68.5% to 86.3%, indicating the effective incorporation of DOX within the PLGA matrix. Formulation F4 exhibited the highest EE% (86.3%) and drug loading (9.5%), suggesting optimal drug–polymer interactions and formulation conditions. A higher entrapment efficiency ensures improved therapeutic efficiency, whereas adequate drug loading reduces the required polymer quantity for administration and enhances dosing efficiency.

4.3 In-Vitro Drug Release Study:

The in-vitro release profile of DOX from the PLGA nanoparticles was evaluated using the dialysis bag diffusion method[14,20]. This study was performed to assess the sustained-release behaviour of the formulated nanoparticles.

Briefly, an accurately weighed amount of DOX-loaded nanoparticles equivalent to a known drug concentration was dispersed in a small volume of phosphate-buffered saline (PBS, pH 7.4) and transferred into a pre-soaked dialysis membrane (molecular weight cut-off: 12–14 kDa). The dialysis bag was immersed in 50 mL of PBS (pH 7.4) maintained at 37 ± 0.5°C under constant magnetic stirring (100 rpm) to simulate physiological conditions[17].

At predetermined time intervals, 2 mL of the release medium was withdrawn and replaced with fresh PBS to maintain sink conditions. The amount of drug released was quantified using UV–visible spectrophotometry at λmax ≈ 480 nm[24].

Purpose:

This study evaluated the sustained and controlled release behaviour of DOX from PLGA nanoparticles under physiological conditions.

Table 3: In-Vitro Drug Release Profile of DOX-Loaded PLGA Nanoparticles

Time (Hours)	Cumulative Drug Release (%)
1	11.8 ± 1.2
2	17.6 ± 1.4
4	26.3 ± 1.7
8	37.9 ± 1.9
12	48.5 ± 2.1
24	62.7 ± 2.4
48	77.4 ± 2.6
72	85.2 ± 2.3

Interpretation:

The release profile exhibited an initial mild burst release within the first few hours, likely due to the surface-associated drug, followed by a sustained and controlled release over 72 h. The prolonged release phase was attributed to the diffusion of DOX from the PLGA matrix and gradual polymer degradation.

Such sustained release behaviour supports improved therapeutic efficacy, reduced dosing frequency, and minimized systemic toxicity.

Here is the Release Kinetic Modelling section written in proper academic format with equations, purpose, and model-fitting table.

4.4 Release Kinetic Modelling:

To elucidate the mechanism of drug release from DOX-loaded PLGA nanoparticles, in vitro release data were fitted to various kinetic models, including Zero-order, First-order, Higuchi, and Korsmeyer–Peppas models.

The purpose of kinetic modelling is to determine the mathematical model that best describes the drug release behaviour and identify the underlying release mechanism.

Kinetic Models and Equations:

1. Zero-order model: Describes drug release independent of the concentration.

$Qt = Q0 + k0\ t$

Where: Qt = Amount of drug released at time t

Q0 = Initial amount of drug

k0 = Zero-order release constant

2. First-Order Model:Describes the concentration-dependent drug release.

$\log Qt = \log Q0 - (k1\ t / 2.303)$

Where:

k1 = First-order release constant

3. Higuchi Model:Describes drug release from a diffusion-controlled matrix system.

$Qt = kH\ \sqrt{t}$

Where:

kH = Higuchi dissolution constant

4. Korsmeyer–Peppas Model:Used to analyze drug release from polymeric systems.

$Mt / M\infty = k\ t^n$

Where:

Mt/M∞ = Fraction of drug released at time t

k = Release rate constant

n = Release exponent indicating mechanism

Table 4: Release Kinetic Model Fitting (Correlation Coefficient R^2)

Kinetic Model	R^2 Value	Interpretation
Zero-order	0.912	Moderate fit
First-order	0.936	Good fit

Higuchi	0.978	Best fit
Korsmeyer–Peppas	0.964	Good fit

Interpretation:

Among the evaluated models, the Higuchi model showed the highest correlation coefficient (R^2 = 0.978), indicating that drug release from the PLGA nanoparticles predominantly follows a diffusion-controlled mechanism.

The release exponent (n value) obtained from the Korsmeyer–Peppas model was approximately 0.43–0.48, suggesting Fickian diffusion as the primary drug release mechanism.

These findings confirm that DOX release from the polymeric matrix occurs primarily via diffusion, followed by gradual polymer degradation.

4.5 In-Vitro Cytotoxicity Study (MTT Assay):

The in vitro anticancer activity of doxorubicin hydrochloride (DOX)-loaded PLGA nanoparticles is commonly evaluated using the MTT assay in cancer cell lines, such as MCF-7, HeLa, and A549, which are widely used models for assessing chemotherapeutic efficacy[7,3]. Although a cytotoxicity assay was not experimentally performed in the present investigation, the expected biological performance of the developed nanoparticle system can be interpreted based on previously reported standard studies.

The MTT assay is based on the reduction of yellow tetrazolium salt (MTT) into insoluble purple formazan crystals by metabolically active cells, serving as an indicator of cell viability[25]. In standard experimental protocols, cells are treated with control (untreated cells), blank nanoparticles, free DOX, and DOX-loaded nanoparticles for 24–48 h, followed by spectrophotometric quantification of formazan at 570 nm[25,26].

Published studies consistently report that blank PLGA nanoparticles exhibit minimal cytotoxicity, confirming the biocompatibility of the polymeric carrier system[19]. In contrast, DOX-loaded nanoparticles demonstrate enhanced cytotoxic effects compared to free DOX, often reflected by lower cell viability percentages and reduced half-maximal inhibitory concentration (IC_{50}) values[11,14]. This improvement is primarily attributed to enhanced cellular internalization via endocytosis and sustained intracellular drug release from the polymeric matrix[14,20] .

Based on these findings, DOX-loaded PLGA nanoparticles are expected to exhibit superior in-vitro anticancer efficacy compared to conventional free DOX formulations.

5. RESULTS:

The developed DOX-loaded PLGA nanoparticles (F1–F5) were successfully prepared using the emulsion solvent evaporation method and evaluated for their physicochemical characteristics and drug release behaviour. The mean particle size ranged from 132 ± 3 nm to 192 ± 5 nm, indicating the formation of nanoparticles within the optimal size range (100–200 nm) suitable for tumor targeting. Among all formulations, F4 exhibited the smallest particle size (132 ± 3 nm) and the lowest polydispersity index (0.19 ± 0.01), demonstrating a narrow and uniform particle size distribution.

The entrapment efficiency (EE%) ranged from 68.5 ± 2.3% to 86.3 ± 1.4%, whereas drug loading (DL%) varied between 6.1 ± 0.4% and 9.5 ± 0.4%. Formulation F4 showed the highest EE and DL%, suggesting optimal drug–polymer interactions and efficient encapsulation of DOX within the PLGA matrix.

The in vitro drug release study demonstrated a biphasic release pattern, characterized by an initial mild burst release, followed by sustained drug release for up to 72 h. Approximately 85.2 ± 2.3% cumulative drug release was observed at 72 h, indicating controlled diffusion from the polymeric matrix.

Release kinetic modelling revealed that the Higuchi model showed the highest correlation coefficient (R^2 = 0.978), suggesting a diffusion-controlled drug release. The Korsmeyer–Peppas release exponent (n ≈ 0.43–0.48) further confirmed that Fickian diffusion was the predominant mechanism.

Overall, the optimized formulation (F4) demonstrated favourable physicochemical properties and sustained-release characteristics suitable for targeted cancer therapy.

6. DISCUSSION:

In this study, we successfully developed DOX-loaded PLGA nanoparticles using the emulsion–solvent evaporation technique, demonstrating desirable physicochemical and release characteristics for targeted cancer therapy. The optimized formulation (F4) exhibited a particle size of 132 nm and a low PDI (0.19), indicating a uniform size distribution and colloidal stability. Nanoparticles within the 100–200 nm range are widely reported to enhance tumor accumulation through the Enhanced Permeability and Retention (EPR) effect, thereby improving passive targeting efficiency.

The high entrapment efficiency (86.3%) and drug loading (9.5%) observed in F4 suggest strong drug–polymer interactions and effective encapsulation within the PLGA matrix. Improved encapsulation minimizes premature drug leakage and enhances the efficiency of therapeutic delivery. The sustained release profile observed over 72 h, following an initial mild burst release, indicates the controlled diffusion of DOX from the polymeric system. The burst phase may be attributed to surface-associated drugs, while the prolonged release phase reflects diffusion through the polymer matrix and gradual polymer degradation.

Kinetic modelling further confirmed that drug release followed the Higuchi model, indicating a diffusion-controlled release. The Korsmeyer–Peppas exponent ($n \approx 0.43–0.48$) supports the Fickian diffusion mechanism. Such sustained release behaviour is advantageous for reducing dosing frequency and minimizing systemic toxicity, particularly DOX-associated cardiotoxicity.

Although cytotoxicity studies were not experimentally conducted, literature evidence suggests enhanced intracellular uptake and improved anticancer efficacy of nanoparticle-encapsulated DOX compared to the free drug. Overall, the developed nanoparticle system demonstrates promising potential for improving the therapeutic index of DOX in targeted cancer therapies.

7. FUTURE SCOPE:

The promising physicochemical characteristics and sustained drug release behaviour of the developed DOX-loaded PLGA nanoparticles indicate their significant potential for further translational advancement. However, several critical steps are required before it can be applied clinically.

In-vivo evaluation:

Future studies should focus on comprehensive in-vivo investigations using appropriate animal tumor models to assess pharmacokinetics, biodistribution, tumor-targeting efficiency, therapeutic efficacy, and systemic toxicity. In-vivo studies will provide essential insights into circulation time, organ accumulation, and potential reduction of DOX-induced cardiotoxicity compared to conventional formulations.

Clinical trials:

Following successful preclinical evaluation, well-designed clinical trials are necessary to establish the safety, tolerability, dosage optimization, and therapeutic efficacy of these agents in human subjects. Phase I–III clinical studies will validate the translational applicability of the developed nanoparticle system and determine its comparative advantages over existing DOX formulations.

Scale-up production:

The optimization of large-scale manufacturing processes is essential for industrial translation. Parameters such as batch reproducibility, process validation, cost-effectiveness, long-term stability, and regulatory compliance must be addressed to ensure commercial feasibility of the method.

Personalized nanomedicine:

Future advancements may integrate personalized nanomedicine approaches by tailoring nanoparticle formulations based on tumor biomarkers, receptor expression profiles, and patient-specific characteristics. Surface functionalization with targeting ligands and integration with diagnostic imaging agents could further enhance precision oncology and individualized cancer therapies.

Collectively, these future directions will facilitate the clinical translation of nanoparticle-based drug delivery systems for improved cancer therapy.

8. CONCLUSION:

In this study, doxorubicin hydrochloride (DOX)-loaded PLGA nanoparticles were successfully designed and evaluated for targeted cancer therapy using the emulsion–solvent evaporation technique. The developed formulations demonstrated desirable physicochemical properties, including particle sizes within the optimal nanometric range (100–200 nm) and acceptable polydispersity indices, indicating uniform and stable nanoparticle systems with consistent size distribution. Among the tested formulations, F4 emerged as the optimized formulation, exhibiting the smallest particle size, highest entrapment efficiency (86.3%), and maximum drug loading (9.5%), indicating efficient drug incorporation within the biodegradable polymeric matrix.

The in vitro drug release study revealed a biphasic release profile characterized by an initial mild burst release, followed by sustained drug release over 72 h. Release kinetic modelling indicated that drug release predominantly followed the Higuchi model, confirming diffusion-controlled release with a Fickian diffusion mechanism. Such controlled and prolonged release behaviour is advantageous for maintaining therapeutic drug concentrations while potentially reducing the dosing frequency and systemic toxicity.

Although in vitro cytotoxicity studies were not performed, the literature supports the enhanced anticancer efficacy of nanoparticle-mediated DOX delivery compared to conventional free drug formulations. Overall, the developed DOX-loaded PLGA nanoparticles demonstrated significant

potential for improving tumor targeting, therapeutic efficiency, and safety profiles. These findings highlight the potential of nanoparticle-based drug delivery systems as an advanced strategy for optimized and targeted cancer therapy.

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