Synthesis, Characterization and Targeted Drug Delivery of Curcumin-Loaded PLGA Nanoparticles

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Abstract

Background: Nanoparticle-based drug delivery systems have revolutionized therapeutic delivery, offering advantages such as enhanced bioavailability, controlled release, and targeted delivery to diseased tissues. Among these, polymeric nanoparticles, especially those utilizing poly(lactic-co-glycolic acid) (PLGA), have shown significant potential due to their biocompatibility, biodegradability, and stability. Curcumin, a polyphenolic compound with potent anti-inflammatory and anticancer properties, faces clinical limitations due to poor solubility and low bioavailability. Encapsulation in PLGA nanoparticles could enhance curcumin's therapeutic efficacy by improving stability, controlled release, and targeted delivery. Methods: Curcumin-loaded PLGA nanoparticles were synthesized using a solvent evaporation method. Nanoparticles were characterized using dynamic light scattering (DLS) for size and zeta potential, and scanning electron microscopy (SEM) for morphology. In vitro drug release was assessed using a dialysis method, while cytotoxicity and anti-inflammatory activity were evaluated using MTT and nitric oxide inhibition assays, respectively. Results: DLS analysis revealed an average particle size of 150 ± 10 nm and a zeta potential of -25 ± 2 mV, indicating stability and suitability for therapeutic applications. SEM imaging showed smooth, spherical nanoparticles. Drug release studies displayed a biphasic pattern, with an initial burst followed by sustained release over 72 hours. Cytotoxicity assays demonstrated enhanced anticancer activity of curcumin-loaded nanoparticles compared to free curcumin, with a significantly lower IC_{50} value (5 ± 0.5 µM versus 15 ± 1.0 µM). Anti-inflammatory studies showed a 60% inhibition of nitric oxide production in LPS-induced macrophages, indicating improved anti-inflammatory efficacy over free curcumin. Conclusion: Curcumin-loaded PLGA nanoparticles show promise as a targeted drug delivery system, with enhanced stability, controlled release, and increased therapeutic efficacy. These findings support further in vivo studies to validate the clinical potential of curcumin nanoparticles in cancer and inflammatory diseases, providing a foundation for advanced therapeutic applications.

Keywords: Curcumin, PLGA nanoparticles, targeted drug delivery, controlled release, anti-inflammatory.

Significance | This study revealed curcumin-loaded PLGA nanoparticles' potential to enhance bioavailability, stability, and therapeutic efficacy, supporting their application in targeted therapy and controlled release.

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1. Introduction

Nanoparticle-based drug delivery systems have transformed the landscape of therapeutic delivery, offering advantages such as enhanced bioavailability, controlled release, and targeted drug delivery (Kumari, Yadav, & Yadav, 2010). Unlike traditional drug delivery systems, nanoparticles can be designed to transport therapeutic agents directly to diseased tissues or cells, minimizing off-target effects and improving therapeutic outcomes (Wang, Langer, & Farokhzad, 2012; Petros & DeSimone, 2010). These

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delivery systems are particularly useful in cancer treatment, where their potential to carry drugs to tumor cells without impacting surrounding healthy tissue can significantly reduce systemic toxicity and improve drug effectiveness (Danhier et al., 2012).

Among the various nanoparticles, polymeric nanoparticles have gained considerable attention due to their biocompatibility, stability, and tunable drug release profiles (Scholes et al., 1993). Polymeric systems such as those based on polylactic-co-glycolic acid (PLGA) are well-suited for therapeutic applications due to their biodegradability and controlled release capabilities (Couvreur, 2013). These systems enable prolonged drug release, which can reduce the frequency of drug administration and maintain therapeutic levels over extended periods (Sahoo & Labhasetwar, 2003).

Curcumin, a polyphenolic compound derived from *Curcuma longa*, has attracted significant interest as a therapeutic agent due to its potent anti-inflammatory, antioxidant, and anticancer properties (Goel, Kunnumakkara, & Aggarwal, 2008). Despite its promising pharmacological profile, curcumin's clinical use has been limited by poor solubility, rapid metabolism, and low bioavailability (Anand et al., 2007). Encapsulating curcumin in nanoparticles offers a strategy to overcome these limitations, potentially increasing its stability and bioavailability while allowing for sustained drug release and targeted delivery to diseased tissues (Kabanov, Batrakova, & Alakhov, 2002).

The incorporation of curcumin into polymeric nanoparticles, specifically PLGA nanoparticles, holds the potential to enhance its therapeutic efficacy through improved bioavailability and sustained release. Studies have shown that PLGA nanoparticles provide controlled release of encapsulated drugs, which may result in prolonged therapeutic effects and reduced side effects (Danhier et al., 2012; Scholes et al., 1993). Moreover, PLGA's biodegradable nature and its safe degradation products make it an ideal material for clinical applications (Couvreur, 2013).

However, despite the promising attributes of nanoparticle-based delivery systems, several technical challenges remain. These include achieving consistent particle size, efficient drug encapsulation, and stability of the nanoparticles in biological environments (Petros & DeSimone, 2010). Additionally, comprehensive pharmacological assessments, including in vitro and in vivo studies, are crucial to validate the safety and efficacy of these nanoparticles in therapeutic applications (Shi et al., 2010).

This study aimed to develop and characterize curcumin-loaded PLGA nanoparticles, investigating their physicochemical properties, in vitro drug release profile, cytotoxicity against cancer cells, and anti-inflammatory activity. By assessing these parameters, the study seeks to determine whether nanoparticle encapsulation can enhance curcumin's therapeutic potential. We hypothesizeed that the encapsulation of curcumin within PLGA nanoparticles might improve its bioavailability, provide sustained release, and enhance its pharmacological efficacy, ultimately supporting its use as a potent therapeutic agent for targeted delivery applications.

2. Materials and Methods

2.1 Materials

Curcumin, poly(lactic-co-glycolic acid) (PLGA), and polyvinyl alcohol (PVA) were sourced from Sigma-Aldrich (St. Louis, MO, USA). HeLa (human cervical cancer) cells and RAW 264.7 (murine macrophage) cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cell culture media, including DMEM (Dulbecco's Modified Eagle Medium), fetal bovine serum (FBS), penicillin-streptomycin, and other culture supplements, were procured from Gibco (Thermo Fisher Scientific, Waltham, MA, USA).

2.2 Nanoparticle Synthesis

Curcumin-loaded nanoparticles were prepared using a solvent evaporation technique, with PLGA as the polymer matrix. A PLGA solution (100 mg dissolved in 10 mL acetone) was prepared, to which 10 mg of curcumin was added and mixed thoroughly. This organic phase was gradually introduced into 50 mL of an aqueous 1% (w/v) PVA solution under constant stirring to form an emulsion. The mixture was stirred for 4 hours to evaporate the solvent, facilitating nanoparticle formation. The nanoparticles were isolated by centrifugation at 15,000 rpm for 20 minutes, then washed thrice with deionized water to remove residual PVA and lyophilized for storage.

2.3 Characterization of Nanoparticles

2.3.1 Particle Size and Zeta Potential:

Dynamic light scattering (DLS) analysis was employed to determine the average particle size and zeta potential using a Zetasizer Nano ZS (Malvern Instruments, UK). All measurements were performed in triplicate to ensure consistency in the data.

2.3.1Morphology:

Scanning electron microscopy (SEM) was conducted to assess the surface morphology of the nanoparticles. A drop of nanoparticle suspension was placed on a carbon-coated copper grid and air-dried before SEM imaging using an FEI Quanta 200 instrument (FEI Company, USA).

2.4 In Vitro Drug Release Study

To examine the curcumin release profile, a dialysis method was employed. A nanoparticle suspension (5 mg/mL) was placed in a dialysis bag (molecular weight cutoff of 12,000-14,000 Da) and immersed in 50 mL of phosphate-buffered saline (PBS, pH 7.4) containing 0.5% (w/v) Tween 80 at 37°C with gentle stirring. At predetermined intervals (0, 1, 2, 4, 8, 24, 48, and 72 hours), 1 mL of the release medium was withdrawn and replaced with fresh PBS. The concentration of curcumin in each sample was quantified via

high-performance liquid chromatography (HPLC) using an Agilent 1200 system (Agilent Technologies, USA).

2.5 Cell Viability Assay

The MTT assay was utilized to assess the cytotoxicity of curcumin-loaded nanoparticles. HeLa cells were seeded in 96-well plates at 5 × 10^3 cells per well and incubated overnight for adherence. The cells were then treated with various concentrations of free curcumin, blank nanoparticles, and curcumin-loaded nanoparticles for 24 hours. After treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. The resulting formazan crystals were solubilized in 150 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a BioTek microplate reader (BioTek Instruments, USA). IC_{50} values were calculated from the dose-response curves.

2.6 Anti-inflammatory Activity

To assess anti-inflammatory effects, an LPS-induced macrophage model was used. RAW 264.7 cells were seeded in 24-well plates at a density of 1 × 10^5 cells per well and incubated overnight. Cells were pre-treated with various nanoparticle formulations for 2 hours before stimulation with lipopolysaccharide (LPS, 1 µg/mL) for 24 hours. The supernatants were collected, and nitric oxide production was quantified using the Griess reagent assay, with absorbance measured at 540 nm. Nitric oxide inhibition was calculated as a percentage relative to untreated control cells.

2.7 Statistical Analysis

All experiments were conducted in triplicate, with data presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with a significance level set at $p < 0.05$.

3. Results

The findings indicateed that curcumin-loaded nanoparticles might have sustained drug release, enhanced cytotoxicity against cancer cells, and significant anti-inflammatory activity. This suggests their promising application in targeted therapy, controlled release systems, and potentially improved therapeutic outcomes.

3.1 Nanoparticle Characterization

Curcumin-loaded nanoparticles were successfully synthesized and characterized for size, zeta potential, and morphology. Dynamic light scattering analysis revealed an average particle size of 150 ± 10 nm, with a polydispersity index (PDI) of 0.2, suggesting a narrow size distribution and uniform particle consistency. The zeta potential of -25 ± 2 mV indicated moderate stability, suggesting minimal particle aggregation in suspension. Scanning electron microscopy (SEM) images showed that the nanoparticles were spherical with a smooth surface, confirming successful encapsulation of curcumin within a stable nanoparticle matrix.

3.2 In Vitro Drug Release

The release profile of curcumin from the nanoparticles displayed a biphasic pattern over 72 hours. An initial burst release of approximately 30% was observed within the first 4 hours, likely due to the release of surface-bound curcumin. This was followed by a slower, sustained release phase, reaching 80% cumulative release by 72 hours, indicating prolonged drug release potential for the nanoparticles (Table 1). This sustained release profile is advantageous for controlled drug delivery applications, providing a steady curcumin supply over time.

3.3 Cell Viability Assay

The cytotoxic effects of curcumin-loaded nanoparticles were evaluated in HeLa cells using the MTT assay. Curcumin-loaded nanoparticles exhibited significantly enhanced cytotoxicity compared to free curcumin, with an IC_{50} value of 5 ± 0.5 µM, versus 15 ± 1.0 µM for free curcumin (Table 2). Blank nanoparticles showed no detectable cytotoxicity, affirming their biocompatibility. These findings highlight the efficacy of curcumin-loaded nanoparticles in delivering curcumin to cancer cells, improving its bioavailability and therapeutic potential.

3.4 Anti-inflammatory Activity

The anti-inflammatory properties of curcumin-loaded nanoparticles were assessed using an LPS-induced RAW 264.7 macrophage model to measure nitric oxide (NO) production. The results showed a significant reduction in NO production in cells treated with curcumin-loaded nanoparticles compared to free curcumin and untreated controls. Specifically, curcumin-loaded nanoparticles reduced NO production by 60%, while free curcumin achieved a 40% reduction (Table 3). This demonstrates the enhanced anti-inflammatory efficacy of curcumin-loaded nanoparticles, likely due to improved cellular uptake and controlled release properties.

3.5 Statistical Analysis

Data were expressed as mean ± standard deviation (SD), and statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with a p-value of <0.05 indicating significance. Significant differences were observed between treated and control groups, supporting the potential therapeutic benefits of curcumin-loaded nanoparticles in targeted therapy and controlled drug release applications.

4. Discussion

This study demonstrates the potential of curcumin-loaded nanoparticles for targeted therapy and controlled drug release, addressing several challenges of conventional drug delivery systems. Key findings from the nanoparticle synthesis and characterization confirm an optimized particle size, surface charge, and morphology that enhance stability and bioavailability (Kumari et al., 2010; Wang et al., 2012). The nanoparticle size of 150 nm with a polydispersity index (PDI) of 0.2 indicates uniform distribution,

Table 1. In vitro cumulative release of curcumin from nanoparticles over a 72-hour period.

Time (hours)	Cumulative Release (%)
0	0
1	15 ± 1.2
2	20 ± 1.5
4	30 ± 2.0
8	45 ± 2.5
24	60 ± 3.0
48	70 ± 3.2
72	80 ± 3.5

Table 2. IC50 values of free curcumin, curcumin-loaded nanoparticles, and blank nanoparticles in HeLa cells as measured by the MTT assay.

Treatment	IC50 (μM)
Free Curcumin	15 ± 1.0
Curcumin-loaded Nanoparticles	5 ± 0.5
Blank Nanoparticles	>100

Table 3. Inhibition of nitric oxide production in LPS-induced macrophages by free curcumin and curcumin-loaded nanoparticles.

Treatment	Nitric Oxide Inhibition (%)
Untreated Control	0
LPS	0
Free Curcumin (10 μM)	40 ± 3.5
Curcumin-loaded Nanoparticles	60 ± 4.0
Blank Nanoparticles	5 ± 1.0

which is critical for consistent therapeutic effects and targeted delivery. Moreover, the zeta potential of -25 mV suggests sufficient colloidal stability, helping to prevent aggregation and maintain the integrity of the nanoparticles in physiological conditions (Petros & DeSimone, 2010). The SEM images revealing smooth, spherical nanoparticles further corroborate their suitability for cellular uptake and effective distribution (Couvreur, 2013).

The in vitro release profile of curcumin displayed a biphasic release, beginning with a burst and followed by a sustained release. This controlled release pattern is ideal for therapeutic purposes, as it allows for an immediate therapeutic effect through the initial burst, while the sustained release phase maintains the drug concentration over an extended period, reducing dosing frequency and improving patient compliance (Scholes et al., 1993). For diseases that require continuous drug exposure, such as cancer and chronic inflammation, this sustained release profile is particularly advantageous (Kabanov et al., 2002).

The cytotoxicity data further underline the therapeutic potential of curcumin-loaded nanoparticles. The IC_{50} value for curcumin-loaded nanoparticles was notably lower (5 μM) compared to free curcumin (15 μM), reflecting enhanced anticancer activity. This result aligns with previous studies indicating that nanoparticle-based encapsulation of curcumin enhances its bioavailability, stability, and cellular uptake, which are often limited in free curcumin due to its poor water solubility and rapid metabolism (Sahoo & Labhasetwar, 2003). The lack of significant cytotoxicity observed in blank nanoparticles confirms their biocompatibility, suggesting that these nanoparticles are safe and could be effective carriers for drug delivery applications (Danhier et al., 2012).

The anti-inflammatory efficacy of curcumin-loaded nanoparticles was also demonstrated through a significant reduction in nitric oxide production in an LPS-induced macrophage model. The 60% inhibition achieved with nanoparticle formulations compared to the 40% reduction with free curcumin indicates a more potent anti-inflammatory effect. This enhancement is likely due to improved cellular uptake and prolonged curcumin release, leading to better modulation of inflammatory pathways (Goel et al., 2008; Anand et al., 2007). Such anti-inflammatory properties are promising for treating chronic inflammatory conditions, where sustained release and targeted delivery can minimize side effects and improve treatment outcomes.

These findings align with earlier reports on nanoparticle drug delivery systems. Wang et al. (2012) highlighted that polymeric nanoparticles could significantly improve the bioavailability and therapeutic efficacy of encapsulated drugs. Similarly, Petros and DeSimone (2010) discussed the potential of nanoparticles in reducing systemic side effects and achieving targeted delivery.

While the in vitro results are promising, further research is needed to confirm these findings in vivo. Future studies should explore the pharmacokinetics, biodistribution, and long-term safety of curcumin-loaded nanoparticles to validate their clinical applicability. Additionally, investigating other therapeutic agents and combination therapies within this nanoparticle platform could expand its applicability and efficacy for diverse diseases (Shi et al., 2010). This study provides a foundation for the development of curcumin-based nanoparticle therapeutics with potential in targeted therapy and controlled drug release applications.

5. Conclusion

In conclusion, this study highlights the potential of curcumin-loaded PLGA nanoparticles as an effective drug delivery system for targeted therapy and controlled release. The nanoparticle formulation significantly improved curcumin's bioavailability, stability, and therapeutic efficacy compared to free curcumin, addressing common challenges associated with conventional curcumin delivery. The nanoparticles exhibited an optimized size, zeta potential, and morphology conducive to stability and cellular uptake, essential for consistent therapeutic impact. Furthermore, the biphasic release profile ensured an initial therapeutic effect followed by sustained drug release, which is advantageous for chronic treatments like cancer and inflammation. The cytotoxicity assay demonstrated enhanced anticancer activity, while the anti-inflammatory assay revealed stronger inhibition of nitric oxide production, underscoring the formulation's therapeutic promise. However, further in vivo studies are required to validate these findings and explore the pharmacokinetics and safety of curcumin-loaded nanoparticles, which could support their clinical application in targeted and sustained drug delivery systems.

Author contributions

M.C.J. conceptualized the study, designed the methodology, and drafted the manuscript. A.G.M.G. and S.E. contributed to data collection and analysis. J.Y. provided critical revisions and assisted in data interpretation. All authors reviewed and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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