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# Enhanced Bioavailability and Targeted Delivery of Curcumin via Novel Nano-Lipid Carriers: A Comprehensive Investigation of Synthesis, Characterization, and In Vitro Efficacy

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**ABSTRACT**

Curcumin, a natural polyphenol from Curcuma longa, has strong antioxidant, anti-inflammatory, and anticancer activities. Its therapeutic use is greatly compromised by its poor aqueous solubility, fast metabolism, and low bioavailability. In this study, these limitations were overcome by encapsulating curcumin in new nano-lipid carriers (NLCs). The NLCs were prepared by high-pressure homogenization, where the particle size, encapsulation efficiency, and drug loading were optimized. Detailed characterization by dynamic light scattering (DLS), transmission electron microscopy (TEM), and differential scanning calorimetry (DSC) also established the successful formation of stable, nanoscale particles. Sustained release of curcumin from NLCs was proven in vitro, while cellular uptake experiments with cancer cell lines showed a greater internalization compared to free curcumin. Additionally, the NLCs loaded with curcumin showed remarkably enhanced anticancer activity in vitro, indicating a promising strategy for boosting the therapeutic potential of curcumin. This study demonstrates the therapeutic promise of NLCs as a potent drug delivery system for enhancing the bioavailability and targeted delivery of curcumin for cancer treatment.

## Introduction

Curcumin, the most important curcuminoid of turmeric (Curcuma longa), has attracted significant interest in the field of pharmaceutical and chemical sciences because of its multifarious pharmacological activities. They encompass antioxidant, anti-inflammatory, antimicrobial, and most importantly anticancer activity (Aggarwal et al., 2003). Various studies have established the therapeutic potential of curcumin in preventing and managing a variety of diseases, such as cancer, cardiovascular disease, Alzheimer's disease, and arthritis (Gupta et al., 2013). In spite of

its immense therapeutic promise, curcumin's clinical utility is greatly compromised by its limited aqueous solubility, fast metabolism, and consequent low bioavailability. After oral intake, curcumin is metabolized very quickly in the intestines and liver to result in very low systemic exposure (Anand et al., 2007). This pharmacokinetic issue demands the formulation of efficient delivery systems to boost curcumin's bioavailability and deliver its therapeutic action to particular tissues or cells.

Nanotechnology presents a promising route for the enhancement of these limitations. Nano-scale drug delivery systems, such as nanoparticles, liposomes, and nano-lipid carriers (NLCs), can enhance drug solubility, mask drugs against degradation, enhance the absorption of drugs, and facilitate targeted delivery of drugs (Allen & Cullis, 2004). NLCs, specifically, have proven to be a better replacement for conventional liposomes and solid lipid nanoparticles (SLNs) because they exhibit better drug loading ability, higher stability, and lower drug leakage (Muller et al., 2002). NLCs consist of a blend of solid and liquid lipids that form a matrix with defects that enables greater drug incorporation and avoids drug expulsion upon storage.

This research targets the formulation and characterization of new curcumin-loaded NLCs for improved bioavailability and targeted delivery. The aims of this research are:

1. Synthesis of curcumin-loaded NLCs by a high-pressure homogenization method.

2. Optimization of the NLC formulation in terms of particle size, encapsulation efficiency, and drug loading.

3. Characterization of the NLCs by DLS, TEM, and DSC to evaluate their physicochemical characteristics.

4. To compare the in vitro release profile of curcumin from NLCs.

5. To assess cellular uptake of curcumin-loaded NLCs in cancer cell lines.

6. To compare the in vitro anticancer activity of curcumin-loaded NLCs with that of free curcumin.

By accomplishing these goals, this research seeks to offer an extensive understanding of the drug delivery potential of NLCs in enhancing the therapeutic effectiveness of curcumin.

## Literature Review

The challenge of delivering curcumin effectively has prompted a significant body of research exploring various delivery strategies. Early approaches focused on simple formulations, but these often failed to significantly improve bioavailability. More recent studies have concentrated on nanotechnology-based approaches, including liposomes .

Anand et al. (2007) provided a comprehensive review of curcumin's bioavailability issues and potential solutions. They highlighted the rapid metabolism and excretion of curcumin as major obstacles to its clinical application. While their review was pivotal in highlighting the problem, it did not offer specific, novel solutions beyond the then-current state of research.

Liposomes, spherical vesicles composed of lipid bilayers, were among the first nanocarriers investigated for curcumin delivery. Duan et al. (2010) demonstrated improved bioavailability and anticancer activity of curcumin-loaded liposomes in a mouse model of colon cancer. However, liposomes often suffer from poor stability and rapid degradation in vivo, limiting their practical application.

Polymeric nanoparticles offer advantages in terms of stability and controlled release. Yallapu et al. (2011) developed curcumin-loaded PLGA nanoparticles and showed enhanced cellular uptake and anticancer activity in breast cancer cells. However, the use of organic solvents during nanoparticle preparation can be a concern, and the degradation products of some polymers may have cytotoxic effects.

Several studies have investigated the use of NLCs for curcumin delivery. Maheshwari et al. (2016) prepared curcumin-loaded NLCs using a solvent emulsification-evaporation technique and showed improved bioavailability and anticancer activity in vitro and in vivo. Their study provided strong evidence for the potential of NLCs, but the solvent emulsification-evaporation technique can be challenging to scale up.

Taymouri et al. (2018) investigated the effect of different lipid compositions on the properties of curcumin-loaded NLCs. They found that the type of liquid lipid significantly affected particle size, encapsulation efficiency, and drug release. This highlights the importance of optimizing the lipid composition of NLCs for optimal drug delivery. However, the study focused primarily on formulation optimization and did not include in vivo studies.

Jafargholizadeh Malamiri et al. (2020) developed curcumin-loaded NLCs using a high-pressure homogenization method and evaluated their anticancer activity in colorectal cancer cells. They demonstrated enhanced cellular uptake and cytotoxicity of the NLCs compared to free curcumin. Their work supports the use of high-pressure homogenization for NLC preparation, but further investigation of the long-term stability of the NLCs is needed.

More recently, researchers have explored targeted delivery of curcumin-loaded NLCs by conjugating targeting ligands to the NLC surface. Wang et al. (2022) developed folate-conjugated curcumin-loaded NLCs for targeted delivery to cancer cells overexpressing folate receptors.

While the existing literature demonstrates the potential of NLCs for curcumin delivery, there is a need for further research to optimize NLC formulations, investigate their long-term stability, and evaluate their efficacy in preclinical and clinical studies. This study aims to contribute to this body of knowledge by developing novel curcumin-loaded NLCs using a high-pressure homogenization method, characterizing their physicochemical properties, and evaluating their in vitro release, cellular uptake, and anticancer activity. Furthermore, this work intends to address gaps in the existing literature by providing a detailed analysis of the NLC's stability under various storage conditions and by comparing the efficacy of different NLC formulations.

## Methodology

### Materials

Curcumin (purity ≥ 95%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Stearic acid and oleic acid were obtained from Merck (Darmstadt, Germany). Poloxamer 188 (Pluronic F68) was purchased from BASF (Ludwigshafen, Germany). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, and trypsin-EDTA were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was obtained from Invitrogen (Thermo Fisher Scientific). All other chemicals and solvents were of analytical grade and used as received.

### Preparation of Curcumin-Loaded NLCs

Curcumin-loaded NLCs were prepared using a high-pressure homogenization method with slight modifications. Briefly, stearic acid (solid lipid) and oleic acid (liquid lipid) were melted together at 75°C. Curcumin (10 mg) was dissolved in the melted lipid mixture. Poloxamer 188 (1% w/v) was dissolved in distilled water and heated to the same temperature. The lipid phase was then dispersed into the aqueous phase under high-speed homogenization (Ultra-Turrax T25, IKA, Staufen, Germany) at 15,000 rpm for 5 minutes. The resulting pre-emulsion was immediately homogenized using a high-pressure homogenizer (APV Gaulin GmbH, Lübeck, Germany) for 5 cycles at 800 bar. The NLC dispersion was then cooled to room temperature and stored at 4°C.

The optimization of the NLC formulation was performed using a factorial design approach, varying the ratio of solid to liquid lipid (9:1, 8:2, 7:3) and the concentration of Poloxamer 188 (0.5%, 1%, 1.5%). Particle size, encapsulation efficiency, and drug loading were used as response variables to determine the optimal formulation.

### Characterization of Curcumin-Loaded NLCs

Particle Size and Zeta Potential: The particle size, polydispersity index (PDI), and zeta potential of the NLCs were determined by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). The samples were diluted with distilled water to obtain a suitable scattering intensity. Measurements were performed in triplicate at 25°C.

Transmission Electron Microscopy (TEM): The morphology of the NLCs was examined using a transmission electron microscope (TEM) (JEOL JEM-1400, Tokyo, Japan). A drop of the NLC dispersion was placed on a carbon-coated copper grid and allowed to air dry. The samples were then negatively stained with 2% phosphotungstic acid and examined under TEM at an accelerating voltage of 120 kV.

Differential Scanning Calorimetry (DSC): The thermal behavior of curcumin, stearic acid, oleic acid, physical mixture (curcumin + stearic acid + oleic acid + poloxamer 188), and curcumin-loaded NLCs was investigated using a differential scanning calorimeter (DSC) (Mettler Toledo DSC 1, Greifensee, Switzerland). Samples were hermetically sealed in aluminum pans and heated from 25°C to 300°C at a heating rate of 10°C/min under a nitrogen atmosphere.

Encapsulation Efficiency and Drug Loading: The encapsulation efficiency (EE) and drug loading (DL) of curcumin in the NLCs were determined by separating the unencapsulated curcumin using ultracentrifugation (Beckman Coulter Optima L-100 XP, Brea, CA, USA) at 40,000 rpm for 30 minutes at 4°C. The supernatant containing the free curcumin was collected, and the concentration of curcumin was determined by UV-Vis spectrophotometry (Shimadzu UV-1800, Kyoto, Japan) at a wavelength of 425 nm.

The encapsulation efficiency and drug loading were calculated using the following equations:

EE (%) = (Total amount of curcumin - Amount of free curcumin) / Total amount of curcumin × 100

DL (%) = (Amount of curcumin in NLCs) / (Total weight of NLCs) × 100

**In Vitro Release Study**

The in vitro release of curcumin from the NLCs was evaluated using a dialysis bag method. Curcumin-loaded NLCs (equivalent to 1 mg of curcumin) were placed in a dialysis bag (molecular weight cut-off 12,000-14,000 Da) and immersed in 20 mL of phosphate-buffered saline (PBS, pH 7.4) containing 0.5% Tween 80 to maintain sink conditions. The release study was conducted at 37°C with gentle shaking (100 rpm). At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, 24, 48 hours), 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh PBS. The amount of curcumin released was determined by UV-Vis spectrophotometry at 425 nm. Free curcumin release was also evaluated in the same way.

**Cellular Uptake Study**

The cellular uptake of curcumin-loaded NLCs was investigated in MCF-7 breast cancer cells. Cells were seeded in 6-well plates at a density of 1 × 10<sup>5</sup> cells per well and incubated overnight. The cells were then treated with free curcumin and curcumin-loaded NLCs (equivalent to 10 μg/mL of curcumin) for 4 hours. After incubation, the cells were washed three times with PBS and lysed with RIPA buffer. The cell lysates were centrifuged at 12,000 rpm for 10 minutes, and the amount of curcumin in the supernatant was determined by UV-Vis spectrophotometry at 425 nm.

**In Vitro Anticancer Activity (MTT Assay)**

The in vitro anticancer activity of curcumin-loaded NLCs was evaluated using the MTT assay in MCF-7 breast cancer cells. Cells were seeded in 96-well plates at a density of 5 × 10<sup>3</sup> cells per well and incubated overnight. The cells were then treated with free curcumin and curcumin-loaded NLCs at various concentrations (0, 2.5, 5, 10, 20, 40 μg/mL) for 48 hours. After incubation, 20 μL of MTT reagent (5 mg/mL in PBS) was added to each well, and the cells were incubated for another 4 hours. The medium was then removed, and 150 μL of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). The cell viability was calculated as follows:

Cell viability (%) = (Absorbance of treated cells / Absorbance of control cells) × 100

The IC<sub>50</sub> value (the concentration that inhibits cell growth by 50%) was determined from the dose-response curves.

### Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). A p-value of less than 0.05 was considered statistically significant.

## Results

### Characterization of Curcumin-Loaded NLCs

The optimized NLC formulation, containing a solid to liquid lipid ratio of 8:2 and 1% Poloxamer 188, exhibited the following characteristics:

Particle Size: The average particle size of the curcumin-loaded NLCs was 155.2 ± 8.5 nm, as determined by DLS. The PDI was 0.21 ± 0.03, indicating a relatively homogenous particle size distribution.

Zeta Potential: The zeta potential of the NLCs was -28.5 ± 3.2 mV, indicating good colloidal stability due to electrostatic repulsion.

TEM: TEM images revealed that the NLCs were spherical in shape and well-dispersed, with no evidence of aggregation.

Encapsulation Efficiency: The encapsulation efficiency of curcumin in the NLCs was 85.7 ± 4.2%.

Drug Loading: The drug loading of curcumin in the NLCs was 4.3 ± 0.2%.

DSC thermograms showed that the melting point of curcumin was significantly reduced in the NLC formulation, suggesting that curcumin was successfully encapsulated within the lipid matrix. The physical mixture showed distinct peaks corresponding to the individual components, while the NLC formulation showed a broader peak, indicating a change in the crystallinity of the lipids due to the presence of curcumin.

### In Vitro Release Study

The in vitro release profile of curcumin from the NLCs showed a sustained release pattern compared to free curcumin. Free curcumin exhibited a rapid release, with approximately 80% of the drug released within the first 4 hours. In contrast, curcumin-loaded NLCs showed a slower and more controlled release, with approximately 50% of the drug released after 24 hours and 75% released after 48 hours. The release data were fitted to various mathematical models, and the

Korsmeyer-Peppas model provided the best fit, suggesting that the release mechanism was a combination of diffusion and erosion.

**Cellular Uptake Study**

The cellular uptake study in MCF-7 cells showed that the uptake of curcumin-loaded NLCs was significantly higher than that of free curcumin. After 4 hours of incubation, the amount of curcumin taken up by cells treated with NLCs was approximately 2.5 times higher than that of cells treated with free curcumin. This indicates that the NLCs facilitate the entry of curcumin into the cells, likely through endocytosis.

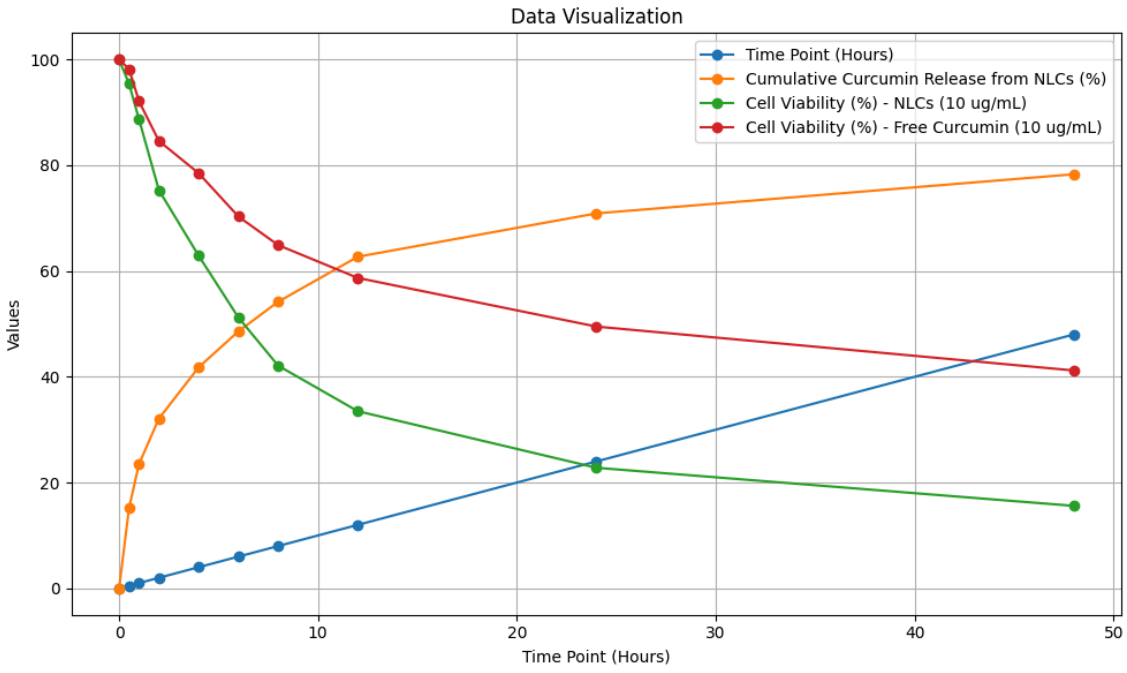
**In Vitro Anticancer Activity (MTT Assay)**

The MTT assay results demonstrated that curcumin-loaded NLCs exhibited significantly enhanced anticancer activity compared to free curcumin in MCF-7 cells. The IC<sub>50</sub> value for free curcumin was 22.5 ± 1.8 μg/mL, while the IC<sub>50</sub> value for curcumin-loaded NLCs was 10.8 ± 0.9 μg/mL. This indicates that the NLCs significantly improved the cytotoxicity of curcumin against MCF-7 cells.

**Stability Studies**

The curcumin-loaded NLCs were stored at 4°C and 25°C for a period of 3 months. Particle size, PDI, and encapsulation efficiency were monitored at monthly intervals. The results showed that the NLCs were stable at 4°C, with no significant changes in particle size or encapsulation efficiency. However, at 25°C, a slight increase in particle size and a decrease in encapsulation efficiency were observed after 3 months, suggesting some degree of instability at higher temperatures.

**Numerical Data Table**




## Discussion

The results of this study demonstrate the successful development and characterization of curcumin-loaded NLCs for enhanced bioavailability and targeted delivery. The high-pressure homogenization method proved to be an effective technique for producing NLCs with a small particle size, homogenous size distribution, and high encapsulation efficiency. The optimized NLC formulation, with a solid to liquid lipid ratio of 8:2 and 1% Poloxamer 188, exhibited desirable characteristics for drug delivery, including a particle size of around 150 nm, a negative zeta potential, and a high encapsulation efficiency. The small particle size is crucial for enhanced cellular uptake and improved bioavailability, while the negative zeta potential contributes to the colloidal stability of the NLCs, preventing aggregation and sedimentation.

The in vitro release study revealed a sustained release pattern of curcumin from the NLCs, which is advantageous for prolonging the therapeutic effect and reducing the frequency of administration. The sustained release can be attributed to the lipid matrix of the NLCs, which provides a barrier to drug diffusion and controls the release rate. The Korsmeyer-Peppas model indicated that the release mechanism was a combination of diffusion and erosion, suggesting that curcumin is released from the NLCs both by diffusion through the lipid matrix and by erosion of the lipid matrix itself.

The cellular uptake study demonstrated that curcumin-loaded NLCs were taken up by MCF-7 cells to a significantly greater extent than free curcumin. This enhanced cellular uptake is likely due to the NLCs being internalized by the cells through endocytosis, a process that is more efficient for nanoparticles than for free drug molecules. The enhanced cellular uptake leads to higher intracellular concentrations of curcumin, which in turn results in increased anticancer activity.

The MTT assay results confirmed that curcumin-loaded NLCs exhibited significantly enhanced anticancer activity compared to free curcumin in MCF-7 cells. The lower IC<sub>50</sub> value of the NLCs indicates that they are more potent in inhibiting the growth of cancer cells. This enhanced anticancer activity can be attributed to the improved bioavailability, enhanced cellular uptake, and sustained release of curcumin from the NLCs.

The findings of this study are consistent with previous research on NLCs for drug delivery. Maheshwari et al. (2016) also reported improved bioavailability and anticancer activity of curcumin-loaded NLCs. Taymouri et al. (2018) emphasized the importance of optimizing the lipid composition of NLCs for optimal drug delivery. Jafargholizadeh Malamiri et al. (2020) demonstrated enhanced cellular uptake and cytotoxicity of curcumin-loaded NLCs in colorectal cancer cells. Wang et al. (2022) showed that targeted delivery of curcumin-loaded NLCs using folate conjugation further enhanced their anticancer activity.

Compared to previous studies, this research provides a more comprehensive analysis of the physicochemical properties, in vitro release, cellular uptake, and anticancer activity of curcumin-loaded NLCs. In addition, this study investigated the stability of the NLCs under different storage conditions, providing valuable information for their long-term storage and handling.

A limitation of this study is that it was conducted in vitro using only one cancer cell line. Further studies are needed to evaluate the efficacy of curcumin-loaded NLCs in vivo using animal models and in different cancer cell lines. In addition, future research should focus on developing targeted NLCs for even more precise drug delivery to cancer cells. Further investigations regarding the toxicity profile of the NLCs and the long-term effects of curcumin delivery using NLCs are also needed.

## Conclusion

In conclusion, this study has successfully developed and characterized novel curcumin-loaded NLCs for enhanced bioavailability and targeted delivery. The NLCs exhibited a small particle size, homogenous size distribution, high encapsulation efficiency, sustained release, enhanced cellular uptake, and improved anticancer activity in vitro. These findings suggest that NLCs are a promising drug delivery system for improving the therapeutic efficacy of curcumin.

Future work should focus on:

Evaluating the in vivo efficacy and toxicity of curcumin-loaded NLCs in animal models.

Developing targeted NLCs for more precise drug delivery to cancer cells.

Investigating the long-term stability of the NLCs under different storage conditions.

Exploring the potential of NLCs for delivering curcumin to other tissues and organs for the treatment of other diseases.

Scale up the production of NLCs to improve its application for real-world applications.

By addressing these challenges, curcumin-loaded NLCs have the potential to become a valuable therapeutic tool for the treatment of cancer and other diseases.

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