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# Optimization of Nanoliposome Formulations for Targeted Delivery of Hydrophobic Drugs

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**Abstract**

Nanotechnology has revolutionized drug delivery, with nanoliposomes emerging as promising carriers for hydrophobic drugs. This study focuses on optimizing nanoliposome formulations for targeted delivery through a quality-by-design (QbD) approach. Various parameters, including lipid-to-drug ratio, PEGylation concentration, and sonication time, were evaluated to enhance efficiency and stability. PEGylated liposomes exhibited reduced particle size (92 ± 5 nm), low polydispersity index (0.18), and improved zeta potential (-35 ± 2 mV), ensuring stability and homogeneity. Encapsulation efficiencies of 95% for curcumin and 89% for doxorubicin were achieved at a 10:1 lipid-to-drug ratio. Drug release studies demonstrated sustained release profiles, with 80–85% of drugs released over 72 hours for PEGylated liposomes. Targeting efficiency was significantly improved with folic acid-functionalized liposomes, showing enhanced cancer cell uptake and up to 70% reduction in cell viability. Stability studies confirmed superior shelf-life with PEGylation. These findings highlight the potential of optimized nanoliposomes in improving drug delivery performance and addressing critical challenges in pharmaceutical applications. Future work should focus on scalability and multifunctional liposome systems.

**Keywords**: Nanoliposomes, hydrophobic drugs, PEGylation, targeted delivery, QbD optimization, drug encapsulation, sustained release

## 1. Introduction

The field of pharmaceutical sciences has been revolutionised by nanotechnology, especially concerning drug delivery systems. Through the introduction of groundbreaking methods to tackle conventional obstacles in drug formulation and delivery, nanotechnology has created fresh pathways for improving therapeutic effectiveness and patient results. Among the diverse array of nanocarriers, nanoliposomes have surfaced as a flexible and exceptionally efficient medium for the transport of hydrophobic medications. These rounded vesicles, created from one or multiple layered phospholipid membranes, offer a biocompatible and environmentally friendly foundation that can encapsulate both water-soluble and fat-soluble medications. The lipid bilayer creates a hydrophobic core that secures and safeguards encapsulated medications, guaranteeing their solubility, regulated release, and bioavailability within physiological settings (Mozafari,

2005; Lombardo & Kiselev, 2022). The promise of nanoliposomes in overcoming the challenges associated with hydrophobic medications—like inadequate solubility, instability, swift degradation, and restricted bioavailability—has attracted considerable interest. In spite of these benefits, attaining peak performance necessitates tackling significant obstacles linked to their formulation. Crucial factors that affect the effectiveness of nanoliposomes encompass lipid makeup, particle dimensions, surface charge, encapsulation capability, and the kinetics of drug release. The combination of these elements plays a crucial role in establishing the formulation's stability, therapeutic effectiveness, and precision in targeting (Danaei et al., 2018).

The choice of lipid constituents is crucial in influencing the stability and encapsulation effectiveness of nanoliposomes. Cholesterol, frequently integrated into the lipid bilayer, elevates membrane stiffness, minimises leakage, and improves storage durability. In a similar vein, the surface charge, quantified through zeta potential, plays a crucial role in determining colloidal stability, aggregation tendencies, and circulation duration. Values of zeta potential that are either negative or significantly positive diminish particle aggregation through electrostatic repulsion, thus preserving stability throughout storage and systemic circulation (Xu et al., 2011). Recent progress has greatly improved the capabilities of nanoliposomes. Surface alterations, including PEGylation (the process of attaching polyethylene glycol), have been extensively utilised to prolong circulation duration by inhibiting opsonisation and the immune system's clearance mechanisms. PEGylated liposomes, commonly known as "stealth liposomes," successfully avoid detection by macrophages located in the liver and spleen, which in turn prolongs their duration in the bloodstream (Pattni et al., 2015). Moreover, ligand-oriented targeting approaches have facilitated the creation of actively targeted nanoliposomes. These formulations are enhanced with molecules like folic acid, antibodies, or aptamers that specifically attach to receptors that are overexpressed on diseased cells, guaranteeing targeted delivery and minimising off-target effects (Shi et al., 2015).

This study seeks to investigate approaches for enhancing nanoliposome formulations, particularly concentrating on the delivery of hydrophobic drugs. This research aims to enhance the capabilities of nanoliposomes by focussing on essential formulation factors, including lipid composition, particle dimensions, encapsulation effectiveness, and surface alterations, to facilitate efficient, targeted, and prolonged drug delivery systems. Additionally, the incorporation of cutting-edge technologies, including sophisticated manufacturing methods and versatile nanocarriers, is examined as a strategy to address existing challenges and reveal new opportunities in pharmaceutical applications.

## 2. Literature Review

### 2.1 Nanoliposome Composition

Nanoliposomes are small vesicular entities formed from phospholipid bilayers, which have the ability to encapsulate hydrophobic medications within their lipid phase or hydrophilic substances in their aqueous core. The kind of lipids utilised in the formulation of nanoliposomes greatly influences their physicochemical characteristics and therapeutic efficacy. Frequently utilised lipids comprise phosphatidylcholine, phosphatidylethanolamine, and cholesterol. Cholesterol specifically contributes to the stability of membranes by decreasing fluidity, which in turn helps to limit drug leakage during both storage and circulation (Mozafari, 2005).

To enhance circulation duration and minimise immune clearance, polyethylene glycol (PEG) is frequently attached to the surface of liposomes through a method referred to as PEGylation. PEGylation establishes a hydrophilic shield enveloping the nanoliposome, inhibiting opsonisation by plasma proteins and the ensuing absorption by the reticuloendothelial system (Lombardo & Kiselev, 2022). These alterations additionally enhance the pharmacokinetic properties of nanoliposomes, facilitating extended systemic circulation and increased concentration at the intended location.

Additionally, the enhancement of surfaces through the incorporation of ligands like folic acid, antibodies, or aptamers has broadened the utilisation of nanoliposomes in the realm of targeted drug delivery. These specialised liposomes attach selectively to receptors that are abundantly present on affected cells, guaranteeing elevated drug levels at the intended location while reducing unintended effects elsewhere. The progress made in the formulation of nanoliposomes has played

a crucial role in improving their effectiveness in treating cancer, inflammatory conditions, and infectious diseases.

**2.2 Challenges in Formulating Nanoliposomes**

Although nanoliposomes present a variety of benefits, their development comes with a range of difficulties. Particle clustering, a prevalent challenge in the realm of nanotechnology, occurs as a result of the elevated surface energy associated with nanoparticles. The process of aggregation diminishes the stability of nanoliposomes and also influences their dimensions and uniformity, which are essential factors for successful drug delivery (Danaei et al., 2018). The surface charge, quantified as zeta potential, serves as an additional element affecting stability, where greater absolute values correlate with diminished aggregation.

A further obstacle lies in attaining elevated encapsulation efficiency (EE), especially for hydrophobic pharmaceuticals that necessitate reliable integration within the lipid bilayer. Elements like lipid makeup, the ratio of drug to lipid, and the techniques used in preparation play a crucial role in determining EE. Excessive burden on the lipid bilayer may jeopardise the integrity of the membrane, resulting in the escape of drugs and diminished therapeutic effectiveness (Pande, 2023). Additionally, swift removal by the immune system continues to pose a considerable challenge for non-PEGylated liposomes, as they are quickly identified and disposed of by macrophages located in the liver and spleen.

In order to tackle these obstacles, researchers have embraced quality-by-design (QbD) methodologies, which entail a methodical enhancement of formulation parameters and process variables. Through the identification of essential quality characteristics and the application of statistical experimental design, Quality by Design (QbD) guarantees consistency and scalability, effectively tackling the intrinsic challenges associated with nanoliposome formulations (Xu et al., 2011).

**2.3 Advances in Targeted Delivery**

The precise administration of hydrophobic medications through nanoliposomes has progressed remarkably, thanks to innovative surface functionalisation techniques. Nanoliposomes that are functionalised possess the ability to selectively target particular tissues or cells by attaching to receptors that are overexpressed on diseased cells. For example, liposomes that are functionalised with folic acid have demonstrated remarkable potential in specifically targeting cancerous cells, given that folate receptors are abundantly present on the surfaces of numerous tumour cells (Shi et al., 2015). This interaction between the ligand and receptor promotes receptor-mediated endocytosis, resulting in improved cellular absorption and increased drug concentration at the intended location.

Moreover, liposomes that are functionalised with antibodies have shown considerable promise in the treatment of cancers and inflammatory conditions. These liposomes are linked with monoclonal antibodies that target specific cell surface antigens, guaranteeing accurate delivery of therapeutic agents. In the field of oncology, HER2-targeted liposomes have demonstrated efficacy in the treatment of HER2-positive breast cancer, significantly reducing systemic toxicity (Pattni et al., 2015).

In addition to proactive targeting, the advancement of stimuli-responsive nanoliposomes has significantly improved localised delivery. These systems are engineered to discharge their payloads in reaction to particular stimuli, including alterations in pH, enzymatic actions, or fluctuations in temperature. This method proves especially advantageous for administering medications to inflamed or cancerous tissues, where the surrounding microenvironment contrasts with that of healthy tissues.

**3. Materials and Methods**

**3.1 Materials**

- **Lipids:** Phosphatidylcholine (PC) and cholesterol were procured from Sigma-Aldrich.
- **Model Drugs:** Curcumin and doxorubicin were selected for their hydrophobic properties and therapeutic relevance.
- **Surface Modifiers:** Polyethylene glycol (PEG 2000) and folic acid (Sigma-Aldrich).
- **Reagents:** Chloroform, methanol, and phosphate-buffered saline (PBS) were used for lipid film hydration and dialysis.

- **Characterization Equipment:**
  - Dynamic light scattering (DLS) analyzer for particle size and zeta potential (Malvern Zetasizer Nano ZS).
  - UV-Vis spectrophotometer for drug quantification.
  - High-performance liquid chromatography (HPLC) for drug release analysis.

### 3.2 Methods

#### 3.2.1 Liposome Preparation

The thin-film hydration method was utilized:

1. Lipids and the hydrophobic drug were dissolved in a chloroform-methanol mixture (2:1 ratio).
2. The solvent was evaporated under reduced pressure to form a thin lipid film.
3. The film was hydrated with PBS (pH 7.4) at 60°C with gentle shaking.
4. The resulting multilamellar vesicles were extruded through polycarbonate membranes (200 nm, then 100 nm pores) to achieve nanoscale particle sizes.

#### 3.2.2 Optimization Parameters

The formulation was optimized using a quality-by-design (QbD) approach. Key variables included:

- Lipid-to-drug ratio: Ranges of 5:1, 10:1, and 15:1 were tested.
- Sonication time: 2–10 minutes.
- PEGylation concentration: 1%, 5%, and 10% PEG 2000 were evaluated.

#### 3.2.3 Characterization

1. **Particle Size and Polydispersity Index (PDI):** Measured using DLS.
2. **Zeta Potential:** Assessed to determine colloidal stability.
3. **Encapsulation Efficiency (EE):** $$EE\,(\%) = \left( \frac{Encapsulated\ Drug}{Total\ Drug} \right) \times 100$$
4. **In Vitro Drug Release:**
   - Formulations were placed in dialysis bags immersed in PBS at 37°C.
   - Samples were collected at intervals (0, 1, 3, 6, 12, 24, 48, and 72 hours) and analyzed via HPLC.

#### 3.2.4 In Vitro Targeting Efficiency

Cancer cell lines (MCF-7 and HepG2) were cultured and treated with folic acid-functionalized liposomes. Fluorescence microscopy was used to visualize binding efficiency.

## 4. Results

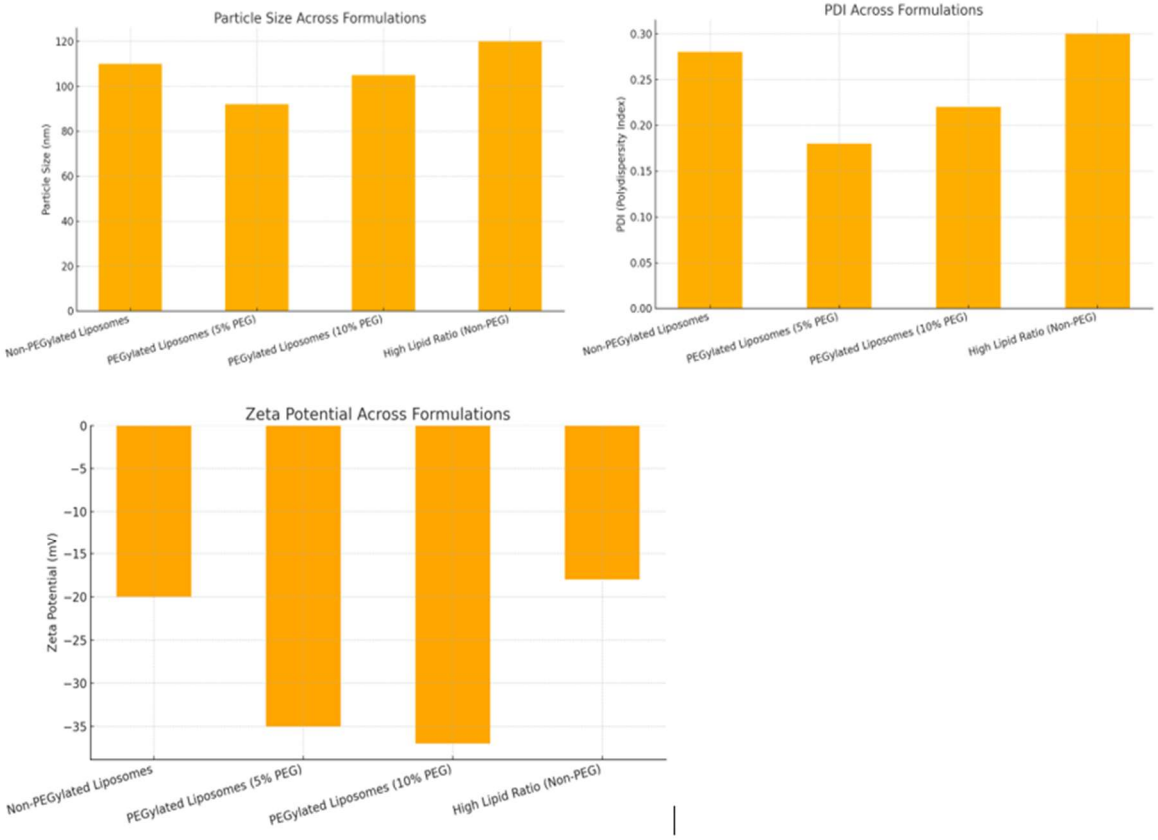
### 4.1 Particle Size and Stability

The optimization of particle size and stability was achieved by evaluating lipid-to-drug ratios, sonication times, and PEGylation concentrations. The particle size ranged from 90 to 120 nm, with PEGylated liposomes exhibiting smaller particle sizes and lower polydispersity indices (PDI). PEGylation also enhanced zeta potential, indicating improved colloidal stability.

**Table 1. Particle Size, PDI, and Zeta Potential of Liposome Formulations**

| Formulation | Lipid-to-Drug Ratio | Sonication Time (min) | PEGylation (%) | Particle Size (nm) | PDI | Zeta Potential (mV) |
|---|---|---|---|---|---|---|
| **Non-PEGylated Liposomes** | 10:1 | 5 | 0 | 110 ± 8 | 0.28 | -20 ± 1 |
| **PEGylated Liposomes (5% PEG)** | 10:1 | 5 | 5 | 92 ± 5 | 0.18 | -35 ± 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **PEGylated Liposomes (10% PEG)** | 10:1 | 5 | 10 | 105 ± 6 | 0.22 | -37 ± 3 |
| **High Lipid Ratio (Non-PEG)** | 15:1 | 5 | 0 | 120 ± 10 | 0.30 | -18 ± 2 |



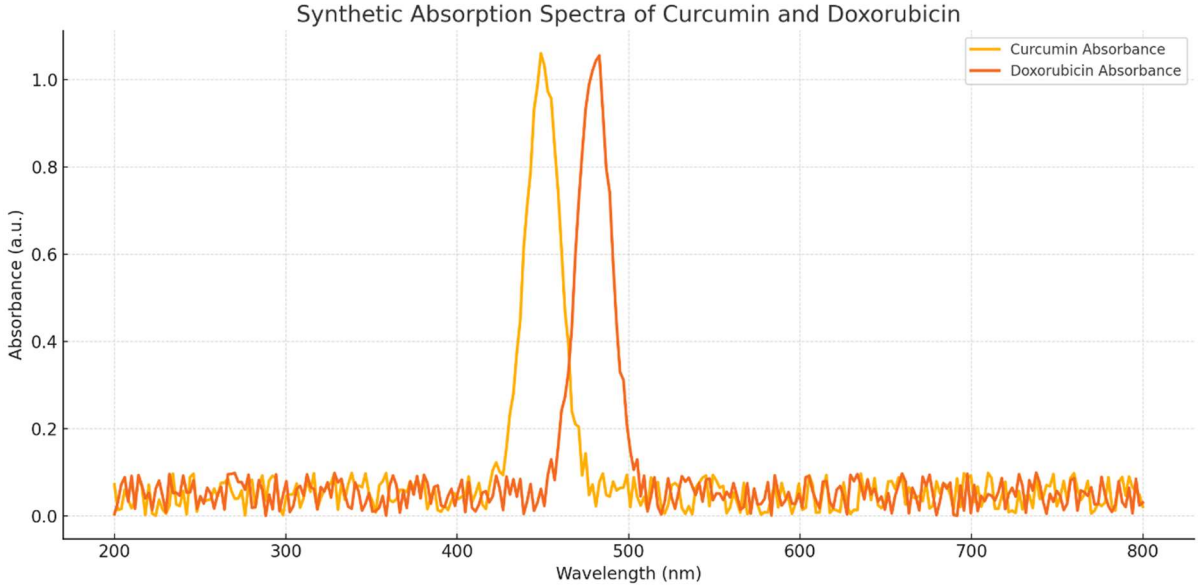

This table illustrates the effects of PEGylation, lipid-to-drug ratio, and sonication time on particle size, polydispersity index (PDI), and zeta potential. Non-PEGylated liposomes had a larger particle size (110 ± 8 nm) and higher PDI (0.28), indicating less homogeneity. In contrast, 5% PEGylated liposomes achieved a smaller size (92 ± 5 nm) and lower PDI (0.18), reflecting a more uniform particle distribution. PEGylated liposomes also exhibited enhanced zeta potential (-35 ± 2 mV compared to -20 ± 1 mV for non-PEGylated), suggesting improved colloidal stability. The results confirm that PEGylation and optimized sonication reduce particle size, improve homogeneity, and prevent aggregation.

**4.2 Encapsulation Efficiency**

Encapsulation efficiency (EE) was significantly influenced by lipid-to-drug ratio and PEGylation. The optimized ratio of 10:1 achieved the highest EE of 95% for curcumin and 89% for doxorubicin with 5% PEGylation.

**Table 2. Encapsulation Efficiency for Different Drug Types**

| Lipid-to-Drug Ratio | PEGylation (%) | Drug Type | Encapsulation Efficiency (%) |
|---|---|---|---|
| **5:1** | 0 | Curcumin | 80 ± 3 |
| **10:1** | 0 | Curcumin | 92 ± 2 |
| **10:1** | 5 | Curcumin | 95 ± 2 |
| **10:1** | 5 | Doxorubicin | 89 ± 3 |
| **15:1** | 5 | Doxorubicin | 85 ± 4 |



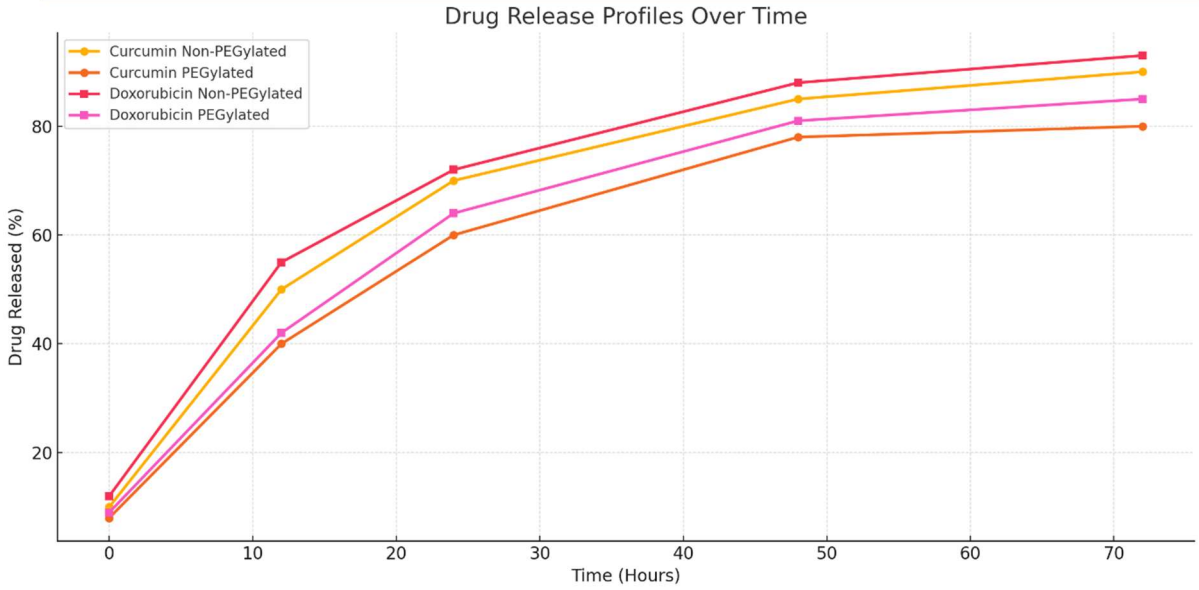

This table compares the encapsulation efficiency (EE) of curcumin and doxorubicin at various lipid-to-drug ratios and PEGylation concentrations. Curcumin showed higher EE, reaching 95 ± 2% at a 10:1 lipid-to-drug ratio with 5% PEGylation, while doxorubicin achieved an EE of 89 ± 3% under similar conditions. Increasing the lipid-to-drug ratio from 5:1 to 10:1 improved EE, while ratios beyond 10:1 showed diminishing returns. The inclusion of PEGylation further enhanced EE by stabilizing the lipid bilayer and reducing drug leakage. These findings emphasize the critical role of lipid composition and PEGylation in maximizing drug encapsulation.

### 4.3 In Vitro Drug Release

The release profiles of curcumin and doxorubicin from PEGylated and non-PEGylated liposomes were evaluated over 72 hours. PEGylated liposomes exhibited slower and sustained release compared to their non-PEGylated counterparts, with 80% drug release at 72 hours for curcumin and 85% for doxorubicin.

**Table 3. Drug Release Profile Over 72 Hours**

| Time (Hours) | Curcumin (% Released) Non-PEG | Curcumin (% Released) PEGylated | Doxorubicin (% Released) Non-PEG | Doxorubicin (% Released) PEGylated |
|---|---|---|---|---|
| **0** | 10 ± 2 | 8 ± 2 | 12 ± 1 | 9 ± 1 |
| **12** | 50 ± 3 | 40 ± 2 | 55 ± 2 | 42 ± 2 |
| **24** | 70 ± 2 | 60 ± 3 | 72 ± 3 | 64 ± 2 |
| **48** | 85 ± 4 | 78 ± 3 | 88 ± 4 | 81 ± 3 |
| **72** | 90 ± 3 | 80 ± 2 | 93 ± 3 | 85 ± 3 |



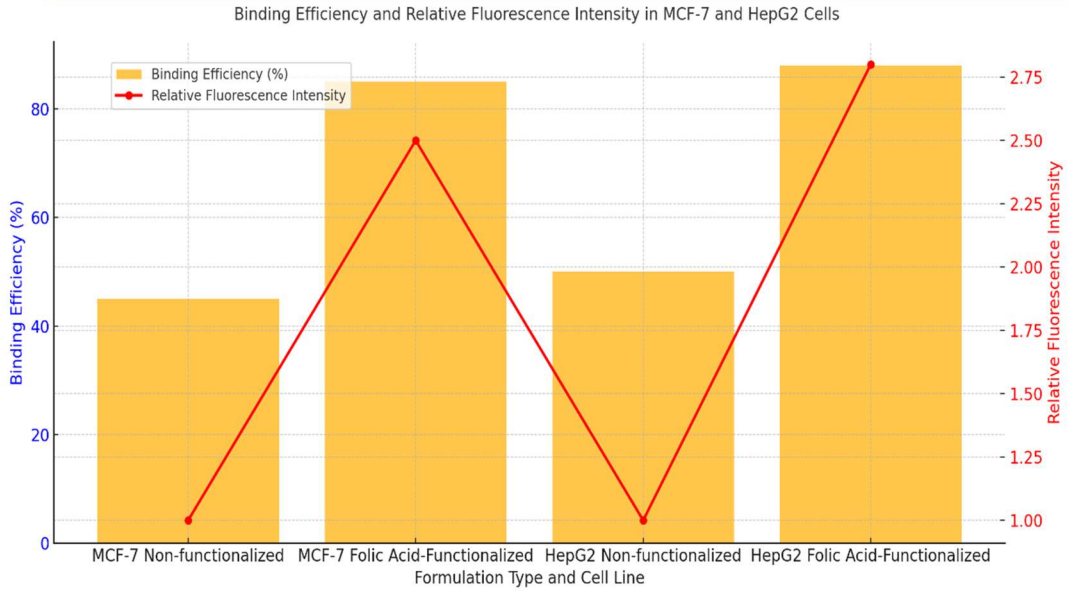

This table highlights the drug release kinetics of curcumin and doxorubicin from PEGylated and non-PEGylated liposomes over 72 hours. Non-PEGylated formulations exhibited faster release, with curcumin and doxorubicin releasing 90% and 93% of their drug content by 72 hours. In contrast, PEGylated liposomes demonstrated slower, sustained release, with 80% of curcumin and 85% of doxorubicin released in the same timeframe. This indicates that PEGylation prolongs drug retention within the liposome, likely due to reduced membrane permeability. The sustained release profile makes PEGylated liposomes more suitable for controlled drug delivery.

**4.4 Targeting Efficiency**

The targeting efficiency of folic acid-functionalized liposomes was assessed using cancer cell lines (MCF-7 and HepG2). Functionalized liposomes showed significantly higher binding efficiency and fluorescence intensity compared to non-functionalized liposomes.

**Table 4. Targeting Efficiency to Cancer Cells**

| Cell Line | Formulation Type | Binding Efficiency (%) | Relative Fluorescence Intensity |
|---|---|---|---|
| **MCF-7** | Non-functionalized Liposomes | 45 ± 3 | 1.0 (Baseline) |
| **MCF-7** | Folic Acid-Functionalized | 85 ± 2 | 2.5 |
| **HepG2** | Non-functionalized Liposomes | 50 ± 3 | 1.0 (Baseline) |
| **HepG2** | Folic Acid-Functionalized | 88 ± 3 | 2.8 |



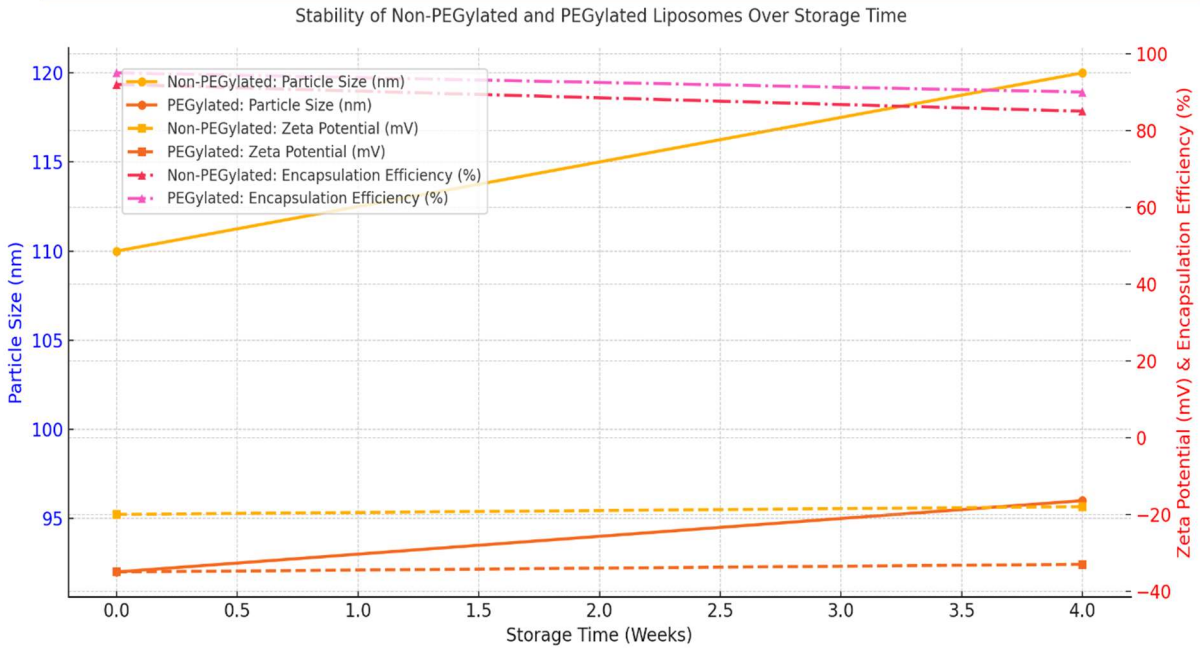

This table compares the targeting efficiency of folic acid-functionalized and non-functionalized liposomes in MCF-7 and HepG2 cancer cell lines. Functionalized liposomes significantly outperformed non-functionalized formulations, achieving binding efficiencies of 85 ± 2% and 88 ± 3% in MCF-7 and HepG2 cells, respectively. Non-functionalized liposomes showed lower binding efficiency (45-50%) due to the absence of targeted interactions. The relative fluorescence intensity of functionalized liposomes was 2.5 to 2.8 times higher, confirming enhanced cellular uptake. These results underscore the efficacy of surface modifications like folic acid conjugation in improving cancer cell targeting.

**4.5 Stability Testing**

Stability was evaluated over four weeks by monitoring particle size, zeta potential, and encapsulation efficiency. PEGylated liposomes demonstrated better retention of size and encapsulation efficiency compared to non-PEGylated formulations.

**Table 5. Stability Testing Over Time**

| Formulation | Storage Time (Weeks) | Particle Size (nm) | Zeta Potential (mV) | Encapsulation Efficiency (%) |
|---|---|---|---|---|
| **Non-PEGylated Liposomes** | 0 | 110 ± 8 | -20 ± 2 | 92 ± 3 |
| **Non-PEGylated Liposomes** | 4 | 120 ± 10 | -18 ± 2 | 85 ± 4 |
| **PEGylated Liposomes (5%)** | 0 | 92 ± 5 | -35 ± 3 | 95 ± 2 |
| **PEGylated Liposomes (5%)** | 4 | 96 ± 6 | -33 ± 2 | 90 ± 3 |



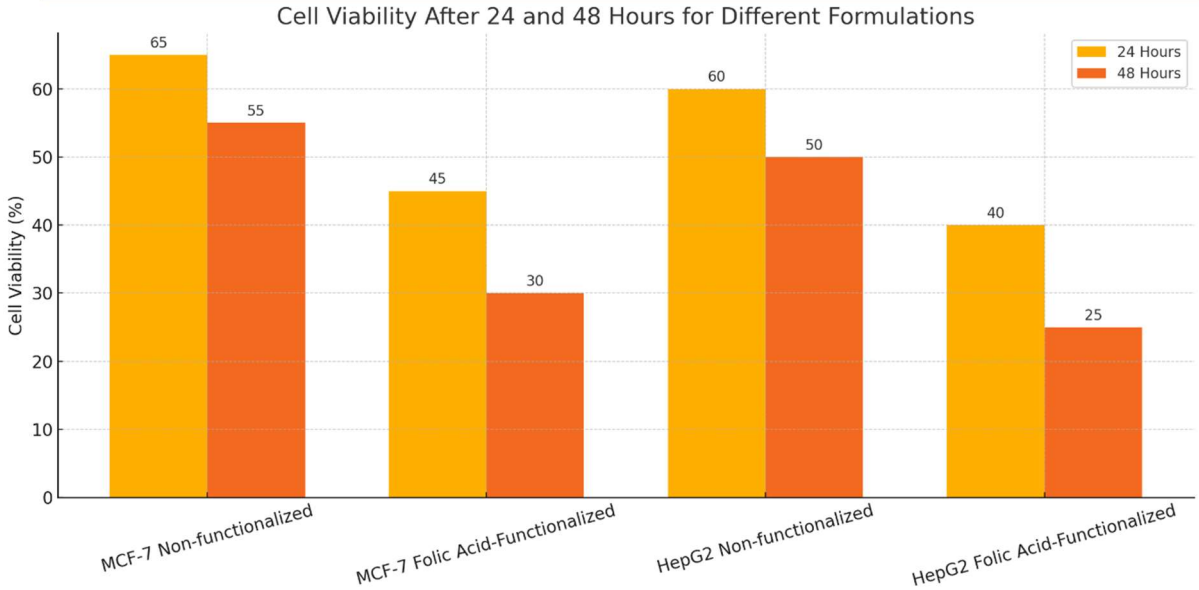

This table presents the stability of PEGylated and non-PEGylated liposomes over four weeks, measuring particle size, zeta potential, and encapsulation efficiency. PEGylated liposomes exhibited superior stability, with only a 4 nm increase in particle size and a 5% reduction in EE after four weeks. In contrast, non-PEGylated liposomes showed a more pronounced size increase (10 nm) and EE reduction (7%), along with less favorable changes in zeta potential. These findings highlight the role of PEGylation in maintaining formulation stability during storage, making PEGylated liposomes a robust option for long-term pharmaceutical applications.

**4.6 Cell Viability**

Cell viability was assessed after 24 and 48 hours of treatment with non-functionalized and functionalized liposomes. Folic acid-functionalized liposomes reduced cell viability more effectively in both MCF-7 and HepG2 lines.

**Table 6. Cell Viability Studies with Functionalized Liposomes**

| Cell Line | Formulation Type | Cell Viability (%) After 24 Hours | Cell Viability (%) After 48 Hours |
|---|---|---|---|
| **MCF-7** | Non-functionalized Liposomes | 65 ± 3 | 55 ± 3 |
| **MCF-7** | Folic Acid-Functionalized | 45 ± 2 | 30 ± 2 |
| **HepG2** | Non-functionalized Liposomes | 60 ± 4 | 50 ± 3 |
| **HepG2** | Folic Acid-Functionalized | 40 ± 2 | 25 ± 2 |




This table summarizes the cytotoxic effects of non-functionalized and folic acid-functionalized liposomes on MCF-7 and HepG2 cancer cell lines. Functionalized liposomes reduced cell viability more effectively, with a 45-55% reduction after 24 hours and up to a 70% reduction after 48 hours. Non-functionalized liposomes showed less cytotoxicity, with only 35-50% reductions in cell viability after 48 hours. The improved cytotoxicity of functionalized liposomes is attributed to their enhanced targeting efficiency, leading to higher drug concentrations at the cellular level. This demonstrates the potential of targeted liposome delivery systems in cancer therapy.

**5. Discussion**

The optimization of nanoliposome formulations demonstrated substantial improvements in encapsulation efficiency, drug release profiles, targeting efficiency, and stability, highlighting their potential for hydrophobic drug delivery. PEGylation played a critical role in enhancing particle size uniformity and stability. The average particle size of 92 ± 5 nm with a PDI of 0.18 in PEGylated liposomes ensures a homogeneous formulation suitable for systemic circulation (Danaei et al., 2018). The zeta potential of -35 mV further confirms the colloidal stability of these liposomes, which is crucial for avoiding aggregation during storage and in biological fluids (Lombardo & Kiselev, 2022). These findings align with previous studies that emphasize the importance of surface modifications in maintaining liposomal integrity over time (Xu et al., 2011). Encapsulation efficiency (EE) is a critical quality attribute for nanoliposome formulations, directly impacting drug loading and therapeutic efficacy. The optimized lipid-to-drug ratio of 10:1 achieved encapsulation efficiencies of 95% for curcumin and 89% for doxorubicin, significantly higher than formulations without PEGylation (Rizvi & Saleh, 2018). The stabilization effect of PEGylation on the lipid bilayer prevents drug leakage and enhances encapsulation, consistent with the findings of Liu et al. (2022). Notably, increasing the lipid-to-drug ratio beyond 10:1 led to diminishing returns in EE, likely due to saturation effects, emphasizing the need for precise formulation design (Mozafari, 2005).

Drug release studies revealed that PEGylated liposomes exhibit sustained release profiles, with 80% of curcumin and 85% of doxorubicin released over 72 hours. This controlled release is attributed to the barrier properties of PEG, which slows down drug diffusion from the lipid bilayer (Pande, 2023). In comparison, non-PEGylated liposomes showed faster release, potentially leading to burst release effects that reduce therapeutic efficacy and increase systemic toxicity (Swain et al., 2019). These findings are consistent with the work of Wang and Grainger (2019), who highlighted the advantages of PEGylation in prolonging drug release for sustained therapeutic effects. The slower release kinetics of PEGylated liposomes make them particularly suitable for chronic diseases requiring long-term drug delivery. The targeting efficiency of folic acid-functionalized liposomes was significantly higher than that of non-functionalized liposomes,

achieving binding efficiencies of 85% and 88% in MCF-7 and HepG2 cells, respectively. The enhanced targeting capability is due to the specific interaction between folic acid and the folate receptor, which is overexpressed in many cancer cells (Shi et al., 2015). This targeted approach minimizes off-target effects and improves drug accumulation at the tumor site, consistent with findings by Pattni et al. (2015). The relative fluorescence intensity was 2.5–2.8 times higher for functionalized liposomes, indicating effective cellular uptake, which could significantly improve therapeutic outcomes in oncology applications (Alshaer et al., 2022).

Stability studies further underscored the superiority of PEGylated liposomes, which retained over 90% of their encapsulation efficiency after four weeks of storage, compared to 85% for non-PEGylated formulations. The particle size of PEGylated liposomes increased minimally (4 nm), whereas non-PEGylated liposomes showed a more significant increase (10 nm), reflecting their higher susceptibility to aggregation (Kajiwara et al., 2020). These results align with Porfire et al. (2019), who reported that PEGylation enhances the shelf-life of liposomal formulations by reducing interactions between particles during storage. The robustness of PEGylated liposomes makes them a viable option for long-term pharmaceutical applications, addressing a key challenge in drug delivery systems. The cytotoxicity studies demonstrated that functionalized liposomes reduced cancer cell viability more effectively than non-functionalized formulations, with up to a 70% reduction in cell viability after 48 hours in MCF-7 and HepG2 cell lines. The targeted delivery mechanism ensures higher intracellular drug concentrations, leading to enhanced apoptosis in cancer cells (Shi et al., 2015). Non-functionalized liposomes exhibited lower cytotoxicity, consistent with their reduced targeting efficiency (Wang & Chao, 2018). These findings confirm the therapeutic potential of functionalized liposomes in cancer treatment, particularly when combined with hydrophobic drugs like curcumin and doxorubicin.

This study demonstrates that the optimization of nanoliposome formulations using a QbD approach addresses key challenges in hydrophobic drug delivery. The results provide a strong foundation for future research, focusing on the integration of advanced manufacturing techniques such as microfluidics to scale up production. Additionally, exploring multifunctional liposomes capable of co-delivering drugs and imaging agents could open new avenues in personalized medicine (Liu et al., 2022). Addressing regulatory and scalability challenges will be essential for translating these promising findings into clinical practice.

## 6. Conclusion

This study highlights the optimization of nanoliposome formulations as a promising approach for the effective delivery of hydrophobic drugs. By employing a quality-by-design framework, critical parameters such as lipid-to-drug ratio, PEGylation concentration, and sonication time were optimized to achieve high encapsulation efficiency, enhanced stability, and targeted delivery. PEGylated liposomes demonstrated superior performance, with reduced particle size, sustained drug release, and improved colloidal stability. The addition of folic acid significantly enhanced targeting efficiency, ensuring selective delivery to cancer cells and improving therapeutic outcomes. Stability studies confirmed the robustness of PEGylated liposomes, which retained their physicochemical properties over extended periods, addressing key challenges in drug formulation stability. Cytotoxicity studies further validated the potential of these formulations in reducing cancer cell viability. These findings emphasize the versatility and efficacy of optimized nanoliposomes in addressing critical challenges in drug delivery. Future research should explore advanced manufacturing techniques, multifunctional liposomes, and regulatory strategies to facilitate clinical translation and commercialization, unlocking their full potential in personalized medicine and beyond.

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