Liposome Nanoparticle: Effect of Process Parameters on Size and Polydispersity

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Abstract

Liposomes are nano-particles widely researched as drug delivery vehicles due to their biocompatibility and ability to encapsulate diverse cargos. Controlling liposome size and polydispersity (non-uniformity of liposome size within a sample) is important for effective delivery, influencing circulation time in the blood and tissue targeting. This study investigates the fabrication of liposomes composed of 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) and cholesterol using the extrusion method. The effects of two key parameters – the number of extrusion passes (5-15) and the DLPC:cholesterol molar ratio (varied from 4:1 up to 19:1) – on final vesicle size and polydispersity index (PdI) were measured using dynamic light scattering (DLS). Results indicated that increasing the number of extrusion passes consistently decreased both the average particle size (from 226.3 nm to 109.8 nm) and the PdI (from 0.368 to 0.082), yielding more homogeneous nanoparticle populations. Varying the DLPC:cholesterol ratio demonstrated a more complex relationship, with an optimal ratio (9:1 DLPC:Cholesterol) producing the smallest and most monodisperse vesicles (123.9 nm, PdI 0.154) under the conditions tested (11 extrusion passes). These findings demonstrate that extrusion parameters significantly impact liposome nanoparticle (LNP) characteristics, providing essential data for optimizing LNP making for potential drug delivery applications. Future work should focus on stability studies and encapsulation efficiency of these formulations.

1 Introduction

Nanoparticles represent an important advancement in medicine, offering platforms for both diagnostics and therapeutics.[1,2] Nanomaterials possess unique physicochemical properties distinct from their bulk counterparts, such as high surface-area-to-volume ratios and tunable surface characteristics, enabling sophisticated biomedical applications.[2] Among various nanoparticle systems, liposomes—spherical vesicles composed of one or more lipid bilayers enclosing an aqueous core—have garnered considerable attention as pharmaceutical carriers.[3,4] Their structural similarity to biological membranes indicate high biocompatibility, and their amphiphilic nature allows for the encapsulation of both hydrophobic drugs within the bilayer and hydrophilic drugs within the aqueous core.[2,3]

Despite their potential, the therapeutic application of liposomes faces challenges, including inherent instability (prone to aggregation and fusion) and rapid clearance from circulation by the reticulo-endothelial system (RES).[1,2,4] Controlling liposome size and achieving a narrow size distribution (low polydispersity) are critical factors in overcoming these limitations. Vesicle size directly impacts circulation half-life, biodistribution, and the ability to extravasate into target tissues, such as tumors, via the enhanced permeability and retention (EPR) effect.[2–4] Consequently, reproducible methods for producing liposomes with defined sizes are essential for developing effective drug delivery systems.

Extrusion, forcing multilamellar vesicles (MLVs) through polycarbonate filters of defined pore sizes, is a widely adopted technique for generating unilamellar vesicles (LUVs) with relatively homogeneous size distributions.[5] This method avoids potentially harmful organic solvents or detergents common in other preparation techniques.[5] The physical properties of the resulting liposomes, including size, lamellarity, stability, and drug retention, are significantly influenced not only by the extrusion process (e.g., pore size, number of passes) but also by the lipid composition.[5] The inclusion of cholesterol, for instance, is known to modulate membrane fluidity, packing density, and stability, which can affect both the formation process and the final vesicle characteristics.[1,4] This study aims to systematically investigate the influence

of two key fabrication parameters: the number of extrusion passes and the molar ratio of 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) to cholesterol, on the resulting liposome size and polydispersity index (PdI), as characterized by dynamic light scattering (DLS). Understanding these relationships is crucial for rationally designing liposome formulations with optimal properties for drug delivery.

2 Background

Liposomes, which are spherical vesicles composed of one of more phospholipid layer surrounding an aqueous core, have been widely used as nanocarriers for drug delivery, cosmetics and gene therapy due to their small size, biocompatibility and ability to encapsulate a wide variety of chemicals.[6] Liposomes were first discovered by Alec Bangham in the 1960s and have since been extensively studied for their ability to increase therapeutic efficacy while minimizing side effects due to ability for targeted delivery and controlled released of active compounds.[3]

Liposome nanoparticles are particularly prevalent in the medical and biotechnology field due to its potential for targeted therapy in cancer treatment or other treatments which requires precise dosage and location in the body. The importance of liposomes comes from their flexibility in formulation, which enable researchers to tailor specific characteristics such as size, membrane permeability and charge.[7] These features affects the kinetics, bioavailibility and biodistributions throughout the body, optimizing the therapeutic effects and minimizing side effects. In addition to medical field, liposomes are also prevalent in the cosmetic industry due to its minute size, which can help active ingredients of products reach deeper penetration of the skin. This can greatly prolong the effects and help reach areas that are difficult to reach.[7]

One advancements in liposome technology is the development of multifunctional liposomes, such as combining drug delivery with diagnostic capabilities, en abling simultaneous treatment and monitoring of disease progression. Together with research on ligand attach-

ment and advancements in liposome engineering, liposomes are continuing to help us advance medicine.[3]

Here, we show the preparation of liposome nanoparticles using extrusion to achieve a consistent and precise distribution of 100 nm particles. The objective of the experiment is to determine the optional process parameters to make 100 nm particles by varying number of extrusions and amount of starting materials. The liposomes are then characterized by DLS to determine the size distribution.

3 Theory

Liposomes are collodial particles that mimic biological membranes and are composed of self-assembled phospholipids. Their fundamental structure consists of one of more lipid bilayers surrounding an aqueous core and the amphillic nature of phospholipids drives the formation of the bilayer, with hydrophobic tails facing inwards and hydrophilic heads facing the aqueous regions.[6] This structure makes it possible for liposomes to encapsulate hydrohpillic substances in their aqueous core and hydrophobic chemicals in within the lipid bilayer, making them very effective in drug deliveries.[8]

Liposomes can vary in size and lamellarity, which can drastically change its properties. Cholesterol can be included in the lipid bilayer to increase membrane durability and reduce leakage, which can help in its longevity in the body.[7] Additionally, surface modifications can help prevent detection by our immune system, hence increasing retention time in our body.[7]

Liposomes are formed spontaneously when phospholipids, which are chemicals with hydrophilic heads and hydrophobic tails, are dispersed in an aqueous solution.[6] This occurs because of hydrophobic interactions resulting in a two layer structure that encapsulates the aqueous phase and are governed by thermodynamic stability. Thin-film hydration is a common and well established method used to make liposomes. First, the lipids are dissolved in a solvent to ensure uniform mixing, then the solvent is evaporated to form a thin lipid film. Water is added

and the dry lipid film spontaneously forms MLVs due to the aforementioned interactions.[8]

Extrusions are a common method to obtain a controlled size distribution in liposome production by forcing the liposome suspension through polycarbonate membranes with a defined pore size.[9] Pushing the MLVs through the pores physically breaks them and they will spontaneously reform into smaller MLVs. After multiple passes through the membrane, the liposomes will have an average size roughly equal to the pore size, giving more accurate and precise distribution.[9]

To determine the size distribution of the nanoparticles, DLS is used, which measures the fluctuations in the intensity of light scattered by the liposomes undergoing Brownian motion in suspension.[10] The scattering of light is measured and analyzed through a correlation function that gives the diffusion coefficient of the particles; smaller particles causes more rapid scattering and diffuses faster when compared to larger particles. The average diameter of the particles can be calculated from diffusion coefficient using the Stokes-Einstein equation

$$D = \frac{k_B T}{6\pi\eta r} \tag{1}$$

where D is the diffusion coefficient, k_B is the Boltzmann constant, T is the absolute temperature, η is the dynamic viscosity of the fluid and r is the radius of the particle.[11] DLS also measures the PdI of the liposomes, which is a measure on the size uniformity within a sample. A low PdI suggests that the the liposomes are uniform and monodispersed.[11]

4 Methods

Liposomes were prepared using the extrusion method. First, a lipid film was created by dissolving DLPC and cholesterol stock solutions in chloroform in a glass vial, followed by gentle shaking to combine. Chloroform was subsequently evaporated under a gentle stream of air to yield a thin lipid film on the vial walls. The lipid film was hydrated by adding 2 mL of deionized water. The suspension was agitated first using a vortex mixer for approximately 30 s, followed

by sonication in a bath sonicator for approximately 2 min. For extrusion, the lipid suspension was loaded into a 1 mL glass syringe. The extruder apparatus was assembled by placing a membrane filter (100 nm pore size) between two spacers within the Teflon housing. The loaded syringe was carefully twisted onto one luer-lok connection of the extruder assembly, and an empty 1 mL glass syringe was connected to the opposite side. The lipid suspension was then extruded by slowly pushing the plunger of the loaded syringe. This process was repeated by pushing the plunger of the now-filled syringe back through the extruder.

Liposomes were prepared with a constant DLPC:Cholesterol composition (9:1 ratio, achieved using 450 μL DLPC stock and 50 μL cholesterol stock) and extruded for varying numbers of passes: 5, 7, 9, 11, 13, and 15 times. In addition, another preparation of Liposomes were made with varying DLPC:Cholesterol ratios (4:1, 5.67:1, 9:1, and 19:1) achieved by adjusting the initial volumes of stock solutions (e.g., 400 μL DLPC/100 μL Chol for 4:1; 425 μL DLPC/75 μL Chol for 5.67:1; 450 μL DLPC/50 μL Chol for 9:1; 475 μL DLPC/25 μL Chol for 19:1), keeping the total lipid concentration notionally similar while maintaining 1.5 mL chloroform and 2 mL hydration volume. All samples in this set were extruded a constant number of times (11 passes). Following extrusion, samples were prepared for Dynamic Light Scattering (DLS) analysis.

5 Results

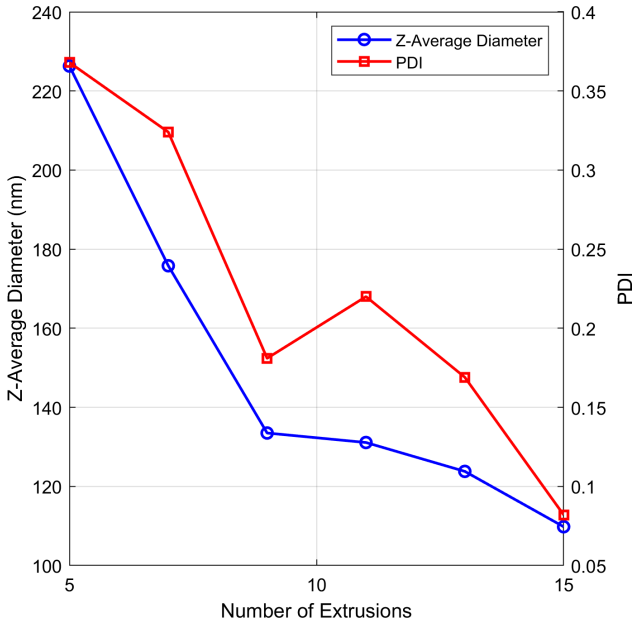

Figure 1: Z-average diameter (left axis, blue) and PdI (right axis, red) of liposome suspensions as a function of the number of extrusions. All samples were prepared with a fixed DLPC:cholesterol molar ratio of 9:1. Z-average diameter and PdI generally decreased with increasing extrusion number, indicating improved size uniformity with additional processing cycles.

Two sets of process parameters were varied to evaluate their effect on liposome size and polydispersity: (1) the number of extrusion passes through a 100 nm membrane, and (2) the molar ratio of DLPC to cholesterol. Liposome samples were characterized using DLS to determine z-average diameter and PdI. Each condition represents a single preparation.

As shown in Fig. 1, increasing the number of extrusion passes from 5 to 15 led to a clear monotonic decrease in both z-average diameter and PdI. The average vesicle size decreased from 226.3 nm at 5 passes to 109.8 nm at 15 passes. In parallel, PdI dropped from 0.368 to 0.082, indicating increasingly uniform vesicle populations with more extrusion cycles. This trend aligns with expectations, as repetitive forcing of MLVs through uniform pore structures promotes reformation into smaller, more homogeneous liposomes. A linear regression of the z-average diameter against extrusion number yielded a slope of –14.6 nm/pass with an R^2 value

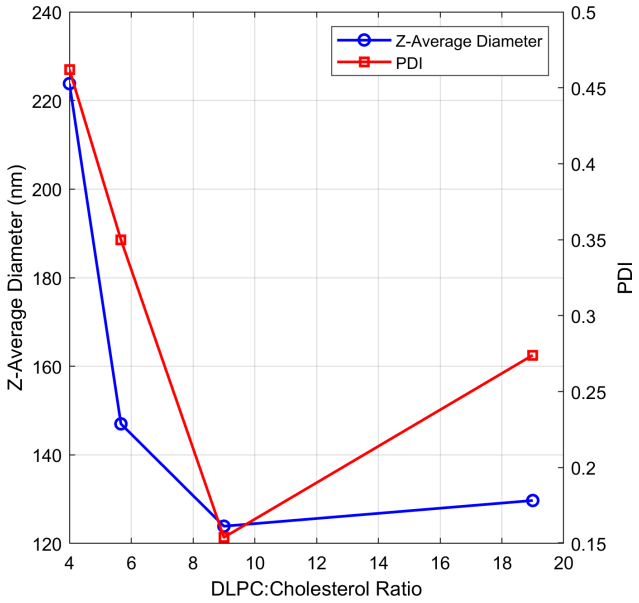

Figure 2: Z-average diameter (left axis, blue) and PdI (right axis, red) of liposome suspensions as a function of DLPC:cholesterol molar ratio. All samples were extruded 11 times. A non-linear trend was observed in both diameter and PdI, suggesting structural changes in the liposome bilayer as cholesterol content varied. Horizontal error bars corresponding to DLPC:cholesterol ratio uncertainty were omitted due to their insignificance relative to the resolution of the x-axis.

of 0.984, supporting a strong linear relationship under the tested conditions.

Fig. 2 displays the impact of varying the DLPC:cholesterol molar ratio while keeping extrusion number constant at 11 passes. The smallest z-average diameter (123.9 nm) and lowest PdI (0.154) were observed at a 9:1 ratio. Both lower (4:1) and higher (19:1) DLPC:cholesterol ratios resulted in larger vesicle sizes (223.8 nm and 129.7 nm, respectively) and higher PdI values (0.462 and 0.274). This non-monotonic behavior suggests a compositional optimum for vesicle formation, where cholesterol balances membrane fluidity and structural integrity. Horizontal error bars for DLPC:cholesterol ratios were omitted due to minimal variability relative to the axis resolution.

Together, these results demonstrate that both extrusion and lipid composition play critical roles in tuning liposome characteristics. Increasing extrusion passes reliably improves size control, while cholesterol content must be optimized to minimize size and polydispersity.

6 Discussion

This experiment explored how both physical processing (extrusion number) and chemical composition (DLPC:cholesterol ratio) influence the size and uniformity of liposome nanoparticles. A consistent downward trend in both z-average diameter and PdI was observed with increasing extrusion number, in agreement with literature precedent that repeated extrusion promotes restructuring of MLVs into smaller, LUVs with narrower size distributions.[12,13] This supports the physical mechanism that extrusion facilitates shearing and breaking of lipid bilayers across pores of uniform size, leading to reduced diameter and enhanced homogeneity.[14]

Importantly, a linear regression analysis revealed a strong inverse correlation between extrusion number and z-average diameter, with an R^2 value of 0.984. This suggests that under controlled conditions, liposome size can be predictably tuned through extrusion count. Further investigation into this linear relationship at lower extrusion numbers could enable the development of predictive models for generating tightly specified nanoparticle sizes. With additional replicates and precise slope determination, this could reduce process trial-and-error and offer pathways for standardized vesicle sizing protocols.

In contrast to the extrusion study, the effect of varying the DLPC:cholesterol molar ratio showed non-monotonic behavior, indicating the presence of a compositional optimal point. The 9:1 ratio resulted in the lowest recorded diameter (123.9 nm) and PdI (0.154), while both lower and higher cholesterol concentrations yielded larger and more polydisperse liposomes. This is consistent with the known role of cholesterol in modulating membrane fluidity and curvature: too little cholesterol can result in flexible, leaky vesicles, while excess may lead to overly rigid structures that resist uniform assembly.[15] The presence of an optimal point highlights that tuning lipid composition is not straightforward and requires careful balancing of compounds used. It also raises the question of what kinds of other compounds, natural or synthetic, can be tested with to produce a more desirable result of 100 nm vesicles with even higher uniformity.

Several limitations must be considered when evaluating the reliability of these findings.

First, leakage from the extrusion device was observed on both days. On Day 2 (Fig. 1 data set), leakage appeared minor and external, with increasing plunger resistance consistent with internal pressure buildup. According to the DLS principles outlined in Eq. (1), this type of volume loss is unlikely to affect measured diameters or PdI. However, leakage was more severe on Day 3 (Fig. 2 data set), and plunger resistance did not increase across extrusion passes. This suggests that some fluid may have bypassed the membrane entirely by flowing directly between syringes. Such crossover would reduce the fraction of MLVs forced through the pores, skewing size distributions upward and producing liposomes that are not fully processed. This could explain the unexpected increase in size and PdI at the highest DLPC:cholesterol ratio. The presence of unextruded particles would broaden the particle size distribution and decrease homogeneity.

Additionally, the number of trials conducted was limited. Due to technical difficulties during the initial setup of the extruder, only a single measurement was obtained for each condition in the given time. This restricts the statistical confidence of the observed trends and precludes formal error analysis. Repeat trials would be essential in future experiments to quantify variability and distinguish systematic effects from noise.

Despite these limitations, the results remain encouraging. The team achieved a minimum z-average diameter of 109.8 nm and a PdI of 0.082, which is within 10% of the target 100 nm size often cited in liposomal drug delivery literature. More significantly, the achieved PdI was over two times smaller than the commonly accepted PdI threshold of 0.2, indicating exceptional size uniformity and suggesting the extrusion protocol was highly effective under optimal conditions.

Future improvements to the procedure could include testing extruder setups with different membrane configurations. For instance, stacking multiple membranes or using different pore diameters may yield tighter size control or reduce the number of extrusion passes needed to reach the target diameter. Additionally, evaluating different membrane materials or suppliers could lower per-run costs or improve durability, making the process more scalable for

industrial applications. Complementing this, the use of sturdier extrusion hardware could reduce leak-based variation and improve reproducibility. Repeating all experiments with multiple replicates would further reinforce the statistical reliability of observed effects and clarify subtle trends.

7 Conclusions

This investigation demonstrated that extrusion parameters critically influence the characteristics of DLPC/cholesterol liposomes. Increasing extrusion passes (5–15) provided effective and predictable control, yielding progressively smaller (109.8 nm minimum) and more uniform (PdI 0.082 minimum) vesicles, aligning with established LUV preparation methods.[5] Concurrently, optimizing the DLPC:Cholesterol ratio proved essential, with a 9:1 ratio producing the most desirable vesicles (123.9 nm, PdI 0.154) under the conditions tested. This non-linear dependence highlights the crucial role of cholesterol in modulating membrane properties during formation.[4] Together, these findings establish conditions (>11 passes, ∼9:1 ratio) for reproducibly fabricating liposomes below 150 nm with low polydispersity (PdI < 0.2). To fully evaluate their therapeutic potential, further studies should address the long-term stability and drug encapsulation efficiency of these optimized formulations.

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Appendix

1. How to Stabilize Phospholipid Liposomes (Using Nanoparticles)

    - Author(s): Liangfang Zhang and Steve Granick
    - Year published: 2006
    - Journal name: Nano Letters
    - 1-3 major accomplishments of this paper:
        (a) Demonstrated stabilization of liposomes against fusion via adsorption of charged nanoparticles.
        (b) Showed stability and lack of leakage at high volume fractions (up to 50%).
        (c) Proposed nanoparticle repulsion as the stabilization mechanism, leaving liposome surface partially free.

2. The antimicrobial activity of liposomal lauric acids against Propionibacterium acnes

    - Author(s): Darren Yang, Dissaya Pornpattananangkul, Teruaki Nakatsuji, et al.
    - Year published: 2009
    - Journal name: Biomaterials
    - 1-3 major accomplishments of this paper:
        (a) Identified lauric acid (LA) as having strong antimicrobial activity against *P. acnes*.
        (b) Demonstrated successful formulation of poorly soluble LA into liposomes (LipoLA).

(c) Showed LipoLA maintained/enhanced antimicrobial activity and likely delivered LA via fusion.

3. Recent advances with liposomes as pharmaceutical carriers

- Author(s): Vladimir P. Torchilin
- Year published: 2005
- Journal name: Nature Reviews Drug Discovery
- 1-3 major accomplishments of this paper:
  (a) Reviewed key developments including PEGylated (long-circulating) and targeted liposomes.
  (b) Summarized clinical progress and challenges in liposomal drug delivery.
  (c) Discussed emerging trends (immunoliposomes, stimuli-sensitive liposomes, gene/protein delivery).

4. Liposome application: problems and prospects

- Author(s): Yechezkel Barenholz
- Year published: 2001
- Journal name: Current Opinion in Colloid & Interface Science
- 1-3 major accomplishments of this paper:
  (a) Reviewed breakthroughs: sterically stabilized liposomes (SSL), remote drug loading, lipoplexes.
  (b) Outlined requirements for therapeutic success (loading, circulation, targeting, release).

(c) Identified remaining challenges (release control, targeting efficiency, cost).

5. Vesicles of variable sizes produced by a rapid extrusion procedure

- Author(s): L. D. Mayer, M. J. Hope and P. R. Cullis
- Year published: 1986
- Journal name: Biochimica et Biophysica Acta
- 1-3 major accomplishments of this paper:

(a) Developed a reproducible extrusion method using polycarbonate filters to create LUVs.

(b) Demonstrated control over final vesicle size by varying filter pore size (30-400 nm).

(c) Showed that combining freeze-thaw with extrusion enhances drug trapping efficiency.

6. Nanoparticles in Medicine: Therapeutic Applications and Developments

- Author(s): L Zhang, FX Gu, JM Chan, et al.
- Year published: 2008
- Journal name: Clinical Pharmacology & Therapeutics
- 1-3 major accomplishments of this paper:

(a) Reviewed advantages of nanoparticle drug delivery (e.g., solubility, half-life, targeting).

(b) Summarized clinically approved nanoparticle drugs (liposomes, polymer conjugates).

(c) Discussed emerging nanoparticle platforms in development.