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Nanoscale Liposome Synthesis for Drug Delivery Applications via Ultrafast Acoustofluidic Micromixing

Ali Pourabdollah Vardin[1,2] **Gurkan Yesiloz**[1,2]
[1] Bilkent University, National Nanotechnology Research Center (UNAM), Ankara, Türkiye
[2] Bilkent University, Institute of Material Science and Nanotechnology, Ankara, Türkiye

ABSTRACT

Nowadays, lipid nanoparticles have gained profound interest in chemical and biomedical engineering. The rapid development of therapeutic nanosystems has led to a need to design suitable approaches to synthesize bio-carriers for efficient drug delivery. Microfluidic methods provide an excellent opportunity to acquire desirable nanoparticle properties, including stability, size, shape, and size distribution, which are often challenging to obtain using conventional bulk synthesis methods. Rapid mixing is a crucial factor in the nanoprecipitation process as it influences the size and size distribution of the nanoparticles. Within this regard, in this work, we report an ultrafast acoustofluidic micromixer to synthesize liposome nanoparticles, which have been widely investigated in the literature as drug carriers due to their biocompatibility and biodegradability. This research has also investigated the influence of glycerol addition to the solvent to control the size of the liposomes. Our findings indicate that utilizing the acoustofluidic platform resulted in the production of nanoscale liposomes with small mean sizes compared to the hydrodynamic flow-focusing (HFF) method. Furthermore, the inclusion of glycerol led to a significant reduction in liposome size. These results emphasize the potential of the proposed approach for the efficient and precise synthesis of liposome nanoparticles with improved characteristics, which can be utilized in various biomedical and drug delivery applications.

Keywords:
Acoustofluidics; Liposome synthesis; Acoustic micromixers; Microfluidics

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***Correspondence to**: Gürkan Yeşilöz,*
E-mail: gurkan@unam.bilkent.edu.tr,
Phone: +90 312 290 8985;
Fax: +90 312 266 4365

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INTRODUCTION

Lipid nanoparticles (NPs) have garnered significant attention in a wide range of chemistry, medicine, and drug delivery studies due to their unique properties [1,2]. Organic nanoparticles are excessively employed as drug carriers in the literature owing to their biocompatibility, biodegradability, and stability [3]. Liposomes are a kind of organic nanoparticle that gain undeniable attention as drug carriers due to their unique characteristics. These nanoscaled spherical structures are composed of a biodegradable lipid bilayer, making them a practical and preferred candidate for delivering drugs. Also, their lipid bilayer structure can encapsulate both hydrophilic and hydrophobic drugs, which makes them versatile vehicles for drug delivery [4-9]. However, in order to leverage these features, liposome nanoparticles need to be produced in a more controlled size and polydispersity manner. The characteristics of nanoparticles, such as size, morphology, and stability, are directly affected by the approach used to synthesize them [10,11]. Conventional bulk synthesis methods often face challenges in generating NPs with controllable size and low polydispersity index (PDI) [12]. Nevertheless, to address these limitations, microfluidic technologies offer an attractive platform for synthesizing NPs with desirable properties, including various sizes and narrower size distributions [13]. Moreover, with microfluidic platforms, multiple operations on the nanoparticles can be done on a single chip, which is not applicable in conventional methods.

Microfluidic-based nanoparticle synthesis can generally be classified into two types: droplet-based (two-phase) and mixing-based (single-phase) microfluidics. Droplet-based microfluidics enables the formation of monodisperse microparticles with larger sizes, providing an opportunity to load them with more drugs or cargo [14,15]. Mixing-based nanoparticle synthesis aims to achieve maximum solvent and solute mixing performance to facilitate homogeneous nanoparticle

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nucleation. This method can be divided into hydrodynamic flow focusing and active micromixers to form nanoparticles. Hydrodynamic flow focusing (HFF) merely relies on diffusion for mixing to produce nanoparticles [16-19]. On the other hand, active methods employ external forces such as acoustic [20,21], electrical [22], or magnetic fields to facilitate the synthesis of nanoparticles. Active micromixers offer rapid and efficient mixing in a shorter time and length, making them an ideal and preferred option for achieving controllable nanoprecipitation and tunable size of nanoparticles (NPs).

In this study, we introduce an ultrafast acoustofluidic micromixer device that enables the production of nanoscale liposome synthesis for drug delivery applications. We utilized a piezoelectric transducer that generates bulk acoustic waves capable of penetrating deep into the material. These generated waves cause oscillatory motion of sharp edges to induce strong microstreaming within the microchannel, consequently reaching higher mixing efficiency. Additionally, the study evaluated the influence of glycerol concentration in the solvent on the liposome nanoparticle properties.

MATERIALS AND METHODS

Materials and Experimental Setup

Glycerol, 98% sn-3-phosphocholine from Sunflower (non-GMO) phospholipid and ethanol were used to synthesize liposome nanoparticles. Also, distilled water and fluorescein dye were utilized for mixing characterization.

The experimental setup used in this work consists of a microfluidic chip integrated with a piezoelectric transducer, a signal generator (Agilent 33220A/ Arbitrary Waveform Generator), a syringe pump (New Era Syringe Pump NE-4000), an amplifier and an inverted microscope (Zeiss, Axio Observer 7) mounted with a CCD camera. The syringe pump allows the injection of fluids at a specific flow rate into the channel. The signal generator produces a square wave signal at the resonance frequency, and this signal's voltage can be increased up to 95 V by the power amplifier. Later, this signal was fed to the piezoelectric transducer, which delivers the acoustic streaming to the microfluidic chip. Moreover, the flow pattern was captured by the inverted microscope and the mounted CCD camera.

Device Fabrication

The standard soft-lithography method was employed to produce microchannels composed of polydimethylsiloxane (PDMS) material with sharp-edge patterns on their sidewalls. The PDMS base was mixed with a curing agent in a ratio of 10:1 and subsequently poured onto the SU-8/ silicon master, which was fabricated in the fashion developed in our previous work [23]. The resulting mold was then cured for a day at room temperature, peeled off from the master, and bonded to a glass substrate using plasma treatment. The channel has a width of 600 μm and a depth of 50 μm, and the sharp edges of the channels have a tip angle of 15°, a length of 225 μm, and a width of 60 μm.

Mechanism of Acoustofluidic Micromixing Platform

As shown in the schematic in Fig. 1, both the PDMS and the piezoelectric transducers are bonded to the glass substrate.

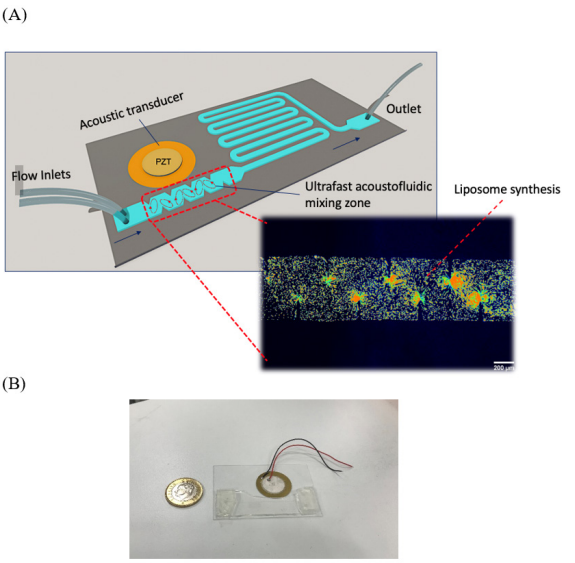

Figure 1. A) Schematic of description of the acoustofluidic micromixer, microstreaming, and the liposome production in the microfluidic platform. B) An image of the acoustofluidic micromixer device.

When the driving voltage is applied to the piezoelectric transducer, the vibration of its membrane is transferred to the PDMS chip, causing the sharp-edge patterns to oscillate. This oscillation generates powerful vortices in the microchannel located near the sharp edges, thereby facilitating the mixing of the two parallel flow streams. The resulting acoustic streaming can enable faster and more efficient mixing, improving liposome nanoparticle synthesis. Overall, the acoustofluidic platform offers a promising tool for enhancing the efficiency and reproducibility of liposome synthesis processes.

Liposome Synthesis and Characterization

To synthesize nanoscale liposomes, a solution of 70% ethanol containing 98% sn-3-phosphocholine was introduced through one inlet of the microfluidic channel, while a mixture of distilled water and glycerol at three different concentrations of 15%, 30%, and 45% were introduced through the other inlet. The injection process was carried out using a syringe pump, and the flow rate ratio of the two inlets was maintained at 1:1. Average diameter

and size distributions of the liposomes were analyzed by a dynamic light scattering (DLS) device (Zetasizer Nano ZS). All the collected samples were loaded into the cuvette and put into the DLS instrument for measurement.

RESULTS AND DISCUSSION

Mixing Characterization

To trace the mixing performance, two fluids with distinct colors were used; diluted fluorescein dye and distilled water. The flow streams were monitored by an inverted microscope. To capture the fluorescence images, we utilized the FITC channel of the microscope with an exposure time of 100 ms. By monitoring the channel with the inverted microscope, visually, it is possible to capture incomplete or complete mixing. However, mixing efficiency has to be obtained numerically to get precise results. Mixing indexes were calculated across the width of the channel based on the following equation [24]:

$$\text{Mixing Index (MI)} = 1 - \frac{\sqrt{\frac{1}{n}\sum\left(I_i - I_{avg}\right)^2}}{I_{avg}}$$

where I_i, I_{avg}, and n are the light intensity value of each point, the average intensity value, and the total number of pixels, respectively. A MI of 0 and 1 indicate unmixed and completely mixed scenarios, respectively.

Effect of the Frequency on Mixing Index

The frequency of a signal is a critical parameter that profoundly impacts the intensity of acoustic streaming. It is essential to determine the resonance frequency of the piezoelectric transducer to achieve optimal efficiency. While every piezoelectric transducer has a unique resonance frequency specified by the manufacturer within a range, it should be determined precisely through visual and quantitative observation during the experiment to locate its exact value to gain better performance. To obtain an accurate resonance frequency, the frequency varied within the range of 3.5-6.5 kHz, with increments of 100 Hz. The captured images were used to calculate the mixing index at the outlet for different frequencies while maintaining a constant total flow rate of 40 µl/min and voltage of 95V. The results revealed that the resonance frequency of the piezoelectric transducer was 4.3 kHz, as depicted in Fig. 2.

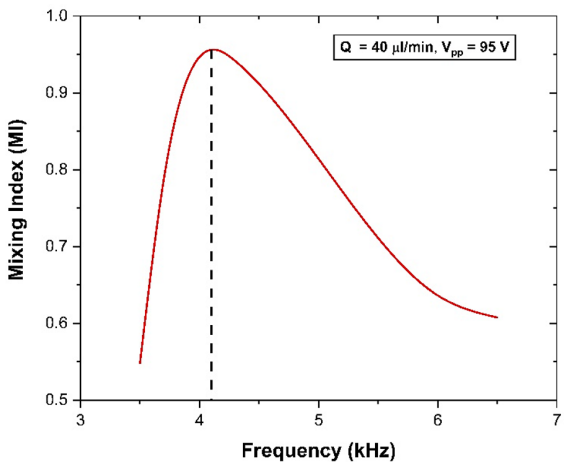

Figure 2. Mixing index at different operating frequencies.

Effect of the Flow Rate and Voltage on the Mixing Index

To explore the impact of flow rate and input voltage on the mixing performance, the frequency was set at 4.3 kHz. The channel was observed under different voltages and flow rates using the inverted microscope, and the resulting images were analyzed to quantify the mixing index. Fig. 3 displays the results, which indicate that at low voltage values (up to 20V) and high flow rates, the acoustic streaming was not sufficiently strong to disrupt the background flow and induce chaotic flow, resulting in incomplete mixing.

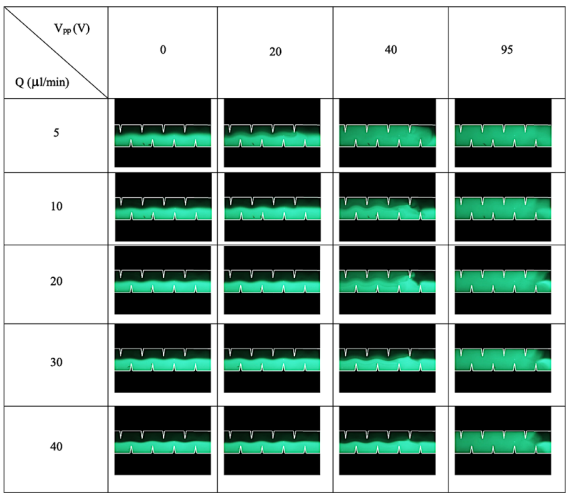

Figure 3. Comparison of mixing performance for different voltages and flow rates.

However, step-wise increasing voltage to higher values, complete mixing was achieved even at high flow rates. Fig. 4 demonstrates that complete mixing occurs for all flow rates when the input voltage is set at 95V. Moreover, it is important to note that since the mixing index varies along the channel, three distinct cross-sectional areas were chosen for the calculation of the mixing index.

Liposome Characterization

The diameter and size distribution of liposome nanoparticles are critical factors for their therapeutic application [25], and these parameters can be controlled by regulating the mixing time during the nanoprecipitation process in microfluidic systems. The proposed acoustof-

luidic system provides direct control over the size of liposomes by adjusting the total flow rate, input voltage, and glycerol percentage in the solvent. Three distinct glycerol concentrations of 15%, 30%, and 45% were utilized.

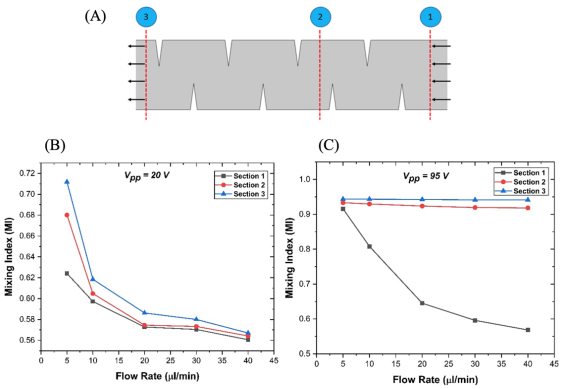

Figure 4. Comparison of mixing performance for different voltages and flow rates.

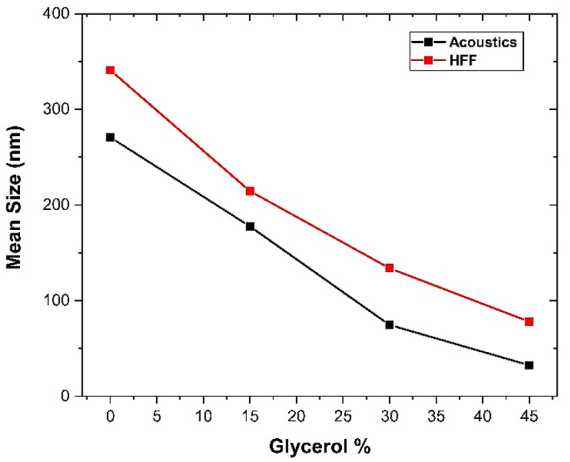

Figure 5. Effect of acoustic micromixer and glycerol percentage on the mean size.

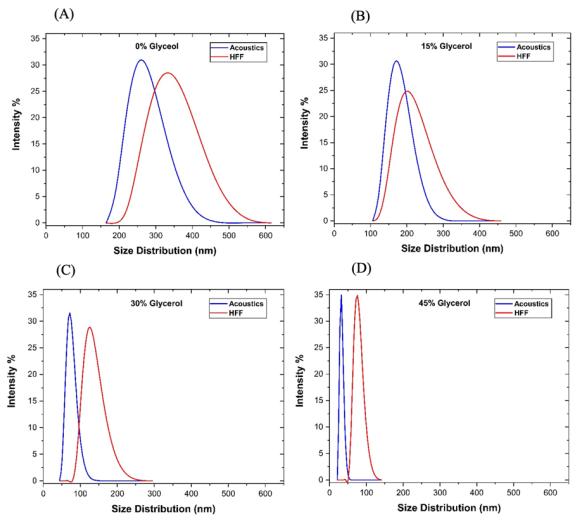

Figure 6. Effect of acoustic micromixer and glycerol percentage on the size distribution (A to D demonstrating the effect of glycerol from 0% to 45%).

To evaluate the size-tuning ability of the acoustofluidic platform, the average diameter of the liposomes was measured using a dynamic light scattering device (DLS) at a total flow rate of 40 µl/min and an input voltage of 95 V for different concentrations of glycerol, with Acoustic and HFF methods. To achieve high throughput, a total flow rate of 40 µl/min was selected as the optimal choice for the synthesis of liposome nanoparticles. As shown in Fig. 5, the mean average size of the liposomes decreased with the implementation of acoustic pressure compared to the HFF method (i.e., in the acoustic-off case). It is worth noting that adding glycerol to the solvent reduced the average size of the liposome nanoparticles, and a greater reduction in the liposome's average diameter was observed with increasing glycerol concentration.

Furthermore, the size distribution of liposomal nanoparticles was analyzed using Dynamic Light Scattering (DLS), as demonstrated in Fig. 6. It can be inferred that the utilization of the acoustic micromixer results in the production of nanoparticles with a lower polydispersity index in comparison to the scenario without acoustic mixing, which indicates its advantageous potential for drug delivery applications.

CONCLUSION

In this study, we report a practical acoustofluidic platform that achieves ultrafast and efficient mixing performance, enabling us to synthesize nanoscale liposome nanoparticles with a high degree of control over particle size compared to the hydrodynamic flow focusing and conventional methods. This acoustofluidic platform leverages sharp-edge patterns that oscillate and vibrate when subjected to acoustic pressure generated by the piezoelectric transducer. This vibration creates strong and powerful vortices in the flow stream, which effectively disturb the fluids, leading to complete mixing in merely a few milliseconds. At the resonant frequency of 4.3 kHz and a voltage of 95 volts, acoustic microstreaming exhibited a remarkable ability to achieve complete mixing of fluids with a total flow rate of 40 µl/min, which is a notably higher flow rate compared to the literature. The results indicate that the implementation of an acoustic micromixer in liposome synthesis resulted in the production of highly uniform nanoparticles with reduced size when compared to the acoustic-off condition. Moreover, the impact of adding glycerol to the solvent was explored, revealing a significant reduction in liposome size upon its inclusion, producing monodispersed liposomes with an average diameter down to 30 nm.

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CONFLICT OF INTEREST

Authors approve that to the best of their knowledge, there is not any conflict of interest or common interest with an institution/organization or a person that may affect the review process of the paper.

AUTHOR CONTRIBUTION

Ali Pourabdollah Vardin performed the experiments and analyzed the results.
Gurkan Yesiloz conceived the idea, fabricated the chip, supervised the experimental progress, analyzed the structure of the work and the results. Both authors drafted and wrote the manuscript.

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