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EXPLORING THE IMPACT OF BUFFER UTILIZATION ON THE PRODUCTION OF CATIONIC LIPOSOMES FOR POTENTIAL BIOLOGICAL APPLICATION

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ABSTRACT

Nanoparticles, the main focus of nanotechnology, have increasingly gained prominence in biological areas, mainly used as nanovehicles. Among the existing nanovehicles, liposomes are the most used. In this sense, this study aims to investigate the influence of phosphate buffer saline (PBS) on the production of cationic liposomes for biological application. The liposomes composed solely of the lipid 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) can be used as a nanocarrier of two other structures, the ovalbumin (OVA) and CpG oligodeoxynucleotides (CpG ODNs), for the development of immunotherapy. The use of the liposome to complex such structures is related to its capacity to help reduce the risk of developing severe allergic reactions, considering that they are biocompatible and capable of offering protection for the cargo. The microfluidic process was adopted to control the nanostructure's physical-chemical characteristics since it is a promising technique for producing lipid nanostructures with high flow control and mixing conditions. In this sense, removing the residual ethanol from liposome formation is crucial since the ethanol in the formulation may cause erythrocyte deformation and become a protein inactivation trigger factor.

Keywords: Liposome. Cationic. Buffer. Immunotherapy.

1 INTRODUCTION

The liposomes, discovered in the 1960s by Bangham [1], are approximately spherical lipid vesicles with sizes around 50 to 500 nm in particle size diameter. These vesicles are formed by specific amphiphilic lipids, natural or synthetic, which self-aggregate into bilayers when in excess of water to reduce the repulsion of the hydrophobic portion of the molecules [2]. Due to the similar structure to the cell membrane, the liposomes are the most used nanovehicles for transporting nucleic acids to cells. Besides, these vesicles are compatible with membrane similarity and are widely applied in medicine and pharmaceuticals [3]. These nanoparticles can be positively, negatively, or zwitterionically charged (negative and positive charge in the same molecule). In addition, substances can be incorporated both into their aqueous interior and between lipid membranes or adsorbed on their surface through electrostatic interactions, depending on the hydrophobicity of such substances [4].

When using nucleic acid and other macromolecules, such as proteins, as therapeutic molecules, one of the difficulties encountered is related to its negative liquid charge that restricts passive diffusion in cells. Cationic liposomes are frequently used to solve this problem as they can carry negatively charged macromolecules [4] through electrostatic interaction. Furthermore, cationic liposomes can be used as adjuvants in vaccines due to their capacity to retain antigens in the vaccine application locally, creating the so-called "deposit effect", which promotes a controlled antigen exposition to immune cells, increasing the vaccine efficacy [5]. In gene therapy, the synthetic lipid 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) is largely used to prepare cationic liposomes.

When considering the utilization of liposomes as carriers for genetic material and other molecules, factors like size and electrical charge significantly influence the outcomes achieved. The production method is one of the main factors affecting liposome's final properties. In this sense, as demonstrated in recent works, microfluidics has been treated and studied as a promising technique to produce lipid nanostructures with high flow control and mixing conditions [6]. From this perspective, this study aims to investigate the influence of phosphate buffer saline (PBS) on the production of cationic liposomes by microfluidic process for biological application. The High-Flow Rate chaotic advection Microfluidic Device (HFR-MD) was used to produce these vesicles since it is a promising technique for achieving high-productivity processes (Figure 1).

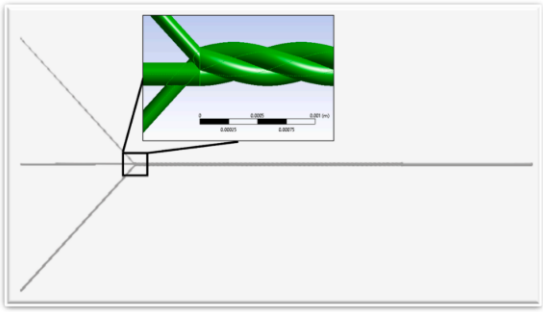

Figure 1 Computer-aided design (CAD) HFR-MD [7].

2 MATERIAL & METHODS

The proposed study was carried out using as materials for the production of liposomes the lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) purchased from Lipoid (Ludwigshafen, Germany), phosphate buffer saline (PBS) (Labormix, Campinas, Brazil), absolute ethanol (99.5%) (Labsynth, São Paulo, Brazil), ultrapure water (ELGA PURELAB® Option-Q) and 3 Å molecular sieve acquired from Sigma-Aldrich. The liposomes were produced by a microfluidic process using a high-flow rate chaotic advection microdevice (HFR-MD). The microfluidic device was manufactured using polydimethylsiloxane (PDMS), needles, and a number 6 urethral probe. Finally, the Amicon Ultra 10 kDa ultrafiltration filter was used to remove the ethanol from the solution with cationic liposomes obtained after the microfluidic process.

The methodology for preparing liposomes using microfluidics begins with the production of the microdevice based on the scaffold method demonstrated by [8]. After this manufacturing stage of the microfluidic device, the lipid nanostructures production protocol begins where, initially, the ethanol is dried in a 3 Å molecular sieve. Then, the DOTAP lipid is dissolved in this solvent at a concentration of 10 mg/mL to create the stock solution used in the process.

Subsequently, the process configuration in which the lateral currents of the microdevice were filled with water or PBS and the central one with the lipid solution, as shown in Figure 2. The nanoparticles were obtained using the Flow Rate Ratio (FRR), the ratio between the lateral and central currents sum equal to 3, and the Total Flow Rate (TFR) of 5. Therefore, the Amicon Ultra ultrafiltration filter was used to remove the ethanol, establishing the centrifugation speed at 9000 G and the temperature at 15 °C.

Dynamic Light Scattering (DLS) was performed to physically characterize the samples produced to obtain information such as average hydrodynamic diameter, size distribution, polydispersity, and Zeta potential.

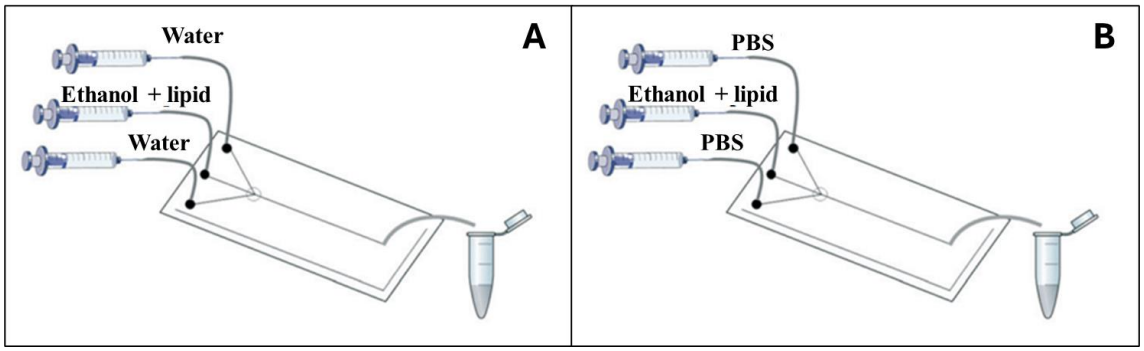

Figure 2 Schematic microfluidic device based on chaotic advection for liposome production shows the configuration used for water (A) and PBS (B) production. Adapted from [8].

3 RESULTS & DISCUSSION

Considering that such liposomes will be intended for biological application for the production of vaccines, ethanol in the formulation can become a triggering factor for the inactivation of active macromolecules, such as proteins [9], and cause erythrocytes deformation into crenated shapes [9]. In this sense, it is necessary to carry out a step after producing these nanostructures to remove the ethanol. The size distribution of the liposomes produced using ultrapure water before and after the ethanol removal process is shown in Figure 3.

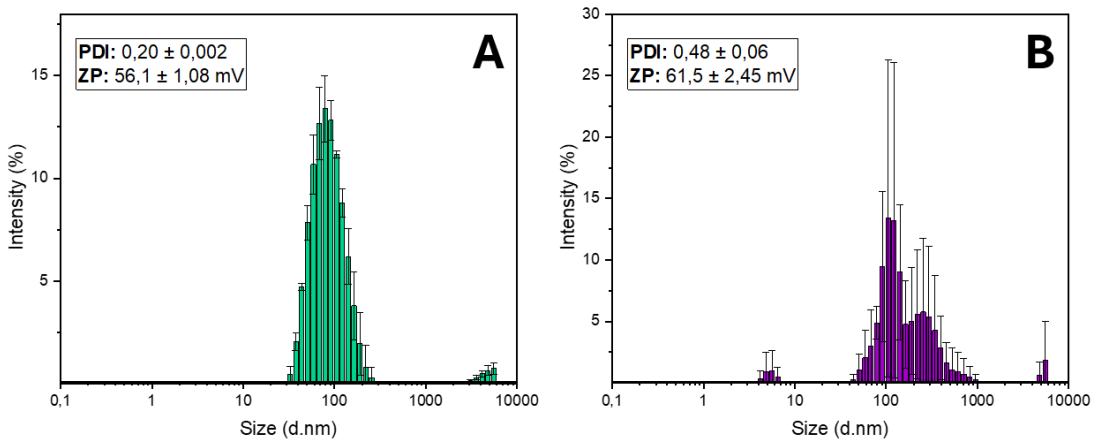

Figure 3 Size distribution in intensity of DOTAP liposomes produced in water before ethanol removal (A) and after ethanol removal (B), with their respective PDIs and zeta potential.

Analyzing the vesicle size distribution before the ethanol removal process, it is possible to observe a monomodal distribution and a low standard deviation. However, when comparing the samples before and after the ethanol removal process, a significant increase in their polydispersity index (from 0.20 ± 0.002 to 0.48 ± 0.06) is observed, as well as an increase in the average particle size (from 79.41 ± 2.19 d.nm to 415.5 ± 175.4 d.nm), resulting in a low-quality reading, and presenting high standard deviations.

Thus, considering the disordered pattern presented in the physicochemical characterization of the sample after removing the ethanol, there was a low stability of the liposomes formed from DOTAP when produced in water, which may be directly related to the fact that the liposomes are very cationic, presenting a zeta potential close to 60 mV. Thus, to study the buffer's influence on the nanostructures' stability, the same process was carried out with samples produced in PBS, and the results can be shown in Figure 4.

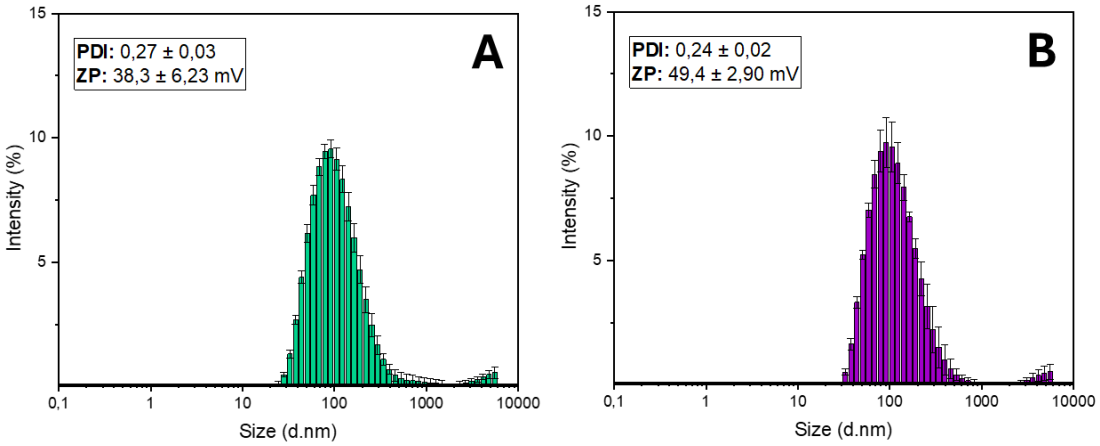

Figure 4 Size distribution in intensity of DOTAP liposomes produced in PBS before ethanol removal (A) and after ethanol removal (B), with their respective PDIs and zeta potential.

The results presented in Figure 3 show a monomodal size distribution both before and after ethanol removal, as well as low PDIs (0.27 and 0.24, respectively) and slight variation in the average particle size, going from 89.81 ± 3.41 d.nm to 98.45 ± 6.28 d.nm. Thus, a sample produced in PBS before and after ultrafiltration to remove ethanol presents greater stability since they possess the same profile and have slight variations in size and polydispersity, which may be related to the high values of zeta potential.

4 CONCLUSION

The results obtained by comparing samples produced in ultrapure water and PBS showed very different behaviors regarding size distribution and polydispersity. While the liposome produced in ultrapure water had a disordered pattern in the distribution size and high deviations after the ethanol-removal process, the one produced in PBS presented greater stability since the size distribution pattern remained the same. The analysis indicates that this phenomenon may be related to the zeta potential, where the particles acquire greater stability in a lower surface charge, as happens when the PBS buffer is used. In this sense, the results showed that the use of buffer to produce the liposomes is viable, making it a promisor to use in the electrostatic formation of complexes for application in vaccines.

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