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# *PLGA Nanoparticle Formation Based on Formulae Incorporating Different Proportions of DOTAP*

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***Abstract:*** The polymer poly(lactic-co-glycolic acid) (PLGA) emulsifies in aqueous conditions to form nanoparticles. Such vesicles are frequently involved in drug delivery, of vaccines for instance, due to several advantages, such as high structural stability, low cytotoxicity and biodegradability. Incorporating lipids onto surfaces of such nanoparticles enhances their effectiveness in cellular internalization. However, the effects on structural integrity in terms of the entire polymer-lipid particle of incorporating lipids onto polymeric nanoparticles is rather less fathomed. In this article we synthesized PLGA vesicles via ultrasonication of suspensions of PLGA as the hydrophobic phase, deionized (DI) water as the inner aqueous phase, and 30% ethanol as the outer aqueous phase. The hydrophobic phase was pre-added 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) in different ratios. The resulting vesicles were presented with scan electron micrographs (SEMs), and were measured their sizes and zeta-potentials. Average zeta potential increased as proportion of lipid to polymer increased, while in all samples there were polydispersion, suggesting the possibility of nanoparticles dissembled. We suggest that the ratio leading to the highest average zeta potential is within the interval between 1:6 and 1:4, or greater than 1:4.

***Keywords:*** Nanoparticle, Stability, Zeta potential, Dispersion

## 1. Introduction

Poly(lactic-co-glycolic acid) (PLGA) is a synthetic polymer which has gathered decent attention in fields of drug development. It is contained in solvents such as ethyl ethanoate, tetrahydrofuran and acetone [1] for protection against hydrolysis of etster bonds. PLGA is frequently involved in drug delivery as the main ingredient of nanoparticles. These particles are formed upon emulsion of suspensions of dissolved PLGA with an aqueous solution, forming nanoparticles that contain the aqueous solution. This can be achieved by a multitude of procedures including single/multiple emulsion processes, with ultrasonication being one of them [1].

Polymeric nanoparticles are less profound than liposomes in performance of drug delivery to cells, as the composition of liposomes are homologous to the cell membrane [2]. In addition, nanoparticles face the obstacle of sedimentation/creaming where smaller particles merge to form greater structures. The two major mechanisms to achieve this are flocculated suspension and deflocculated suspension. In the former, particles form sediment via settling independently, while in the latter, particles aggregate before sedimentation. As a result, surfactants are often incorporated into polymeric nanoparticles to mitigate interactions among particles, with ionic lipids providing electromagnetic

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repulsion being an adopted option [3]. Such system is developed for PLGA nanoparticles with a variety of lipids, in which PLGA with cationic DOTAP is an example with a number of variants such as inclusion of cholesterol. Such systems are used in delivery of polynucleotides, therefore potential carriers for cancer treatment and vaccines [4][5].

Studies have shown that formulations of lipid polymer nanoparticles have different optimal lipid:polymer ratios for percentage yield [6]. Such ratio for optimal stability of DOTAP-PLGA nanoparticles, nevertheless, is less studied. As ionic nanoparticles, stability can be measured via 1) potential difference across the stern layer and the slipping plane, namely zeta-potential 2)degree which nanoparticles exhibit elastic interactions, which can be represented by . In this article we discuss the range of this ratio via 1) Synthesizing lipid polymer nanoparticles and polymeric nanoparticles 2) Qualitative observations of SEMs of each formula 3) Quantitative analysis of the trend of differences in zeta-potentials between lipid polymer formulae with different lipid:polymer mass ratios.

## 2. Materials

PLGA was purchased from Lakeshore Biomaterials, Inc. (Birmingham, Alabama, the USA), DOTAP was purchased from Aladdin-Reagent Co., Ltd (Shanghai, China), anhydrous ethyl ethanoate and anhydrous ethanol were purchased from Beijing Chemical Industry Limited Company (Beijing, China), and DI water was obtained from TTL-68 (Beijing TongTaiLian Technology co., Ltd, Beijing, China).

## 3. Methods

Lipid polymer nanoparticles were prepared via the ultrasonication method. For the sample with DOTAP:PLGA ratio of 1:4, 9mg of PLGA is dissolved in 2mL of anhydrous ethyl ethanoate, and 2.25mg of DOTAP was added to the solution to form the hydrophobic phase. The hydrophobic phase was vortexed and pipetted to 250μg of DI water, the inner aqueous phase, under continuous stirring. After pipetting, the stirring lasted for 8 hours for emulsification. Part of the emulsion was ultrasonicated in ice bath (0°C), at 25W for 10 periods of 4s that were 2s from adjacent periods, and added to 15mL of 30% aqueous ethanol (anhydrous ethanol dissolved in DI water at appropriate amounts, acting as the outer aqueous phase), to form nanoparticles. The nanoparticles were for 2 times, centrifuged at 10000 rpm for 15 minutes, had partial liquid phase decanted off from the residue (condensed nanoparticles), and filled with DI water back to the original mass of the mixture, and the residue dispersed into the residual liquid phase, to remove the most remaining emulsion that failed to form nanoparticles. The resulting liquid was added onto an even aluminum foil in individual droppings, dried via ventilation and had SEMs obtained from the dried nanoparticles on the foil. The same procedure was performed for preparing samples with DOTAP:PLGA ratios of 1:6 and 1:8, but with 1.5mg and 1.125mg of DOTAP respectively.

## 4. Results and Discussion

To measure sample stabilities, nanoparticles were analyzed their zeta-potential distributions and average diameters with ELSZ-2000 (Otsuka Electronics (Suzhou) Co., Ltd.). The mean zeta-potential increased with proportion of DOTAP. All measured average zeta-potentials were positive except for the second measurement for the sample of DOTAP:PLGA ratio 1:8 at -1.64mV.

Figure 2 and Figure 3 show increased average zeta-potential as lipid content in formula increased, which is consistent with the SEMs in Figure 1, which portray progressively clearer boundaries and less adhesion between particles from sample of DOTAP:PLGA=1:8 to 1:4. There exists the outlier, the second trial for sample of DOTAP:PLGA=1:8, with a negative yet small value of -1.64mV.

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Interestingly, most data except for the first trial of DOTAP:PLGA=1:8 are polydispersed, exhibiting 2 peaks each, one significantly more positive than the other, and the more positive (higher) peaks reached higher intensities than their respective more negative (lower) peaks with third trial of DOTAP:PLGA=1:4 as the exception. Only one peak in all 3 trials for DOTAP:PLGA=1:8 is around 0 with the rest being positive for both higher and lower peaks. All trials of the remaining samples each consist of a positive higher peak, although significantly lower than those of DOTAP:PLGA=1:8, and a negative lower peak near the negative limit of the diagram. However, the lower-peaks for DOTAP:PLGA=1:4 and DOTAP:PLGA=1:6 and the higher peaks for DOTAP:PLGA=1:8 are most likely benefited by a minority of nanoparticles in each respective sample considering their trivial contributions to the average zeta potentials, in which those with the more negative lower peaks have in fact the higher average zeta potentials.

As for the exact causes for these unusual peaks, direct assumptions can be made that the lower peaks were from PLGA nanoparticles as they lost their incorporated DOTAP, leaving only anionic [$-COO^-$] groups at terminals of PLGA chains. The higher peaks may have been derived from self-assembly of DOTAP liberated from PLGA, forming cationic liposomes. This theory of liberating lipids could be supported by in which steric stabilization, the act of increasing intermolecular forces in the lipid layer via introduction of hydrophobic compounds into it, is frequently adopted in less polydispersed examples, such as cholesterol [5][7][8], hence removing this process could potentially lead to insufficient molecular interactions, thus increasing likelihood of liberation of nanoparticle compartments. However, the supposed peaks from PLGA nanoparticles seen in DOTAP:PLGA=1:4 and DOTAP:PLGA=1:6 are not seen in DOTAP:PLGA=1:8, nor are peaks from assembled DOTAP assumed for DOTAP:PLGA=1:8 seen in the other samples. An explanation supporting the disappearance of the supposed cationic liposomes in DOTAP:PLGA=1:4 and DOTAP:PLGA=1:6, and one for that of PLGA nanoparticles in ADOTAP:PLGA=1:8 are required to validate the theory. Since the contradiction, it is also possible for the lower peaks to be caused by background noise of analysis.

Hence, we propose that the optimal DOTAP:PLGA ratio for DOTAP-PLGA nanoparticles is within the interval of 1:6 to 1:4, or greater than 1:4, as our experimental data suggests. To further define the interval, nanoparticles with ratios between 1:6 and 1:4, and those with that greater than 1:4, could be tested to determine the trend of zeta potential, and thus stability, of ratios greater or lesser than 1:4.

## 5. Conclusion

The trend of zeta potential in DOTAP-PLGA nanoparticles is proportional to that of the ratio of DOTAP:PLGA, thus indicating increased emulsion stability. However, whether there exists decomposition of such nanoparticles, and the mechanisms responsible if there is, remains to be determined. Future research could focus on the phenomena of polydispersity and its precise causes and mechanisms.

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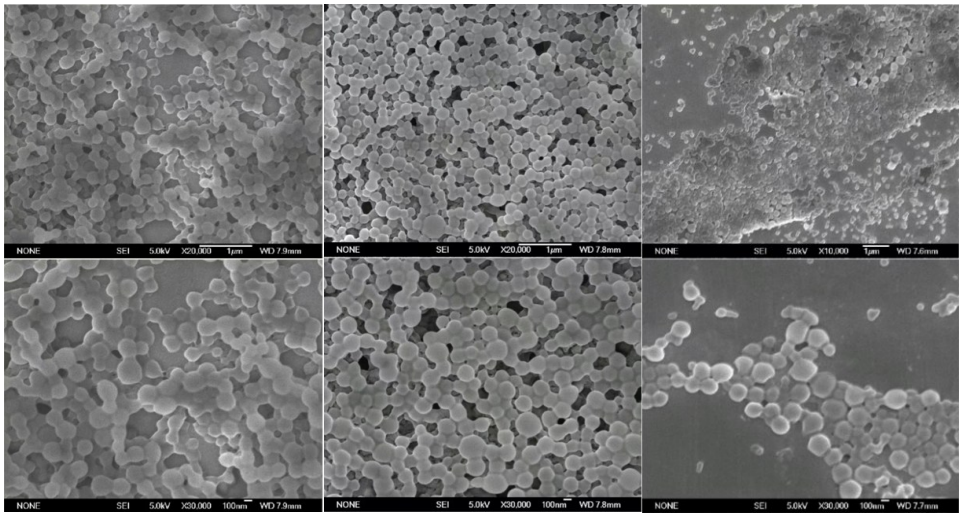

Figure 1: SEMs of DOTAP:PLGA=1:8 (left), DOTAP:PLGA=1:6 (middle) and DOTAP:PLGA=1:4 (right)

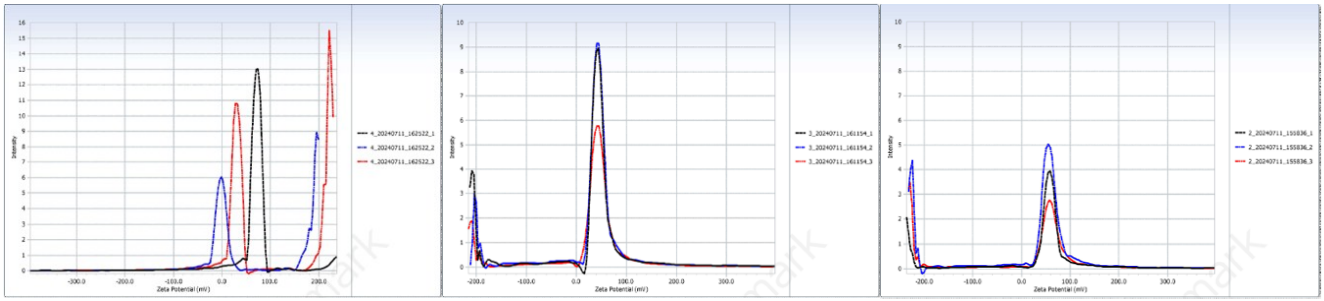

Figure 2: Zeta potential distributions of DOTAP:PLGA=1:8 (left), DOTAP:PLGA=1:6 (middle) and DOTAP:PLGA=1:4 (right)

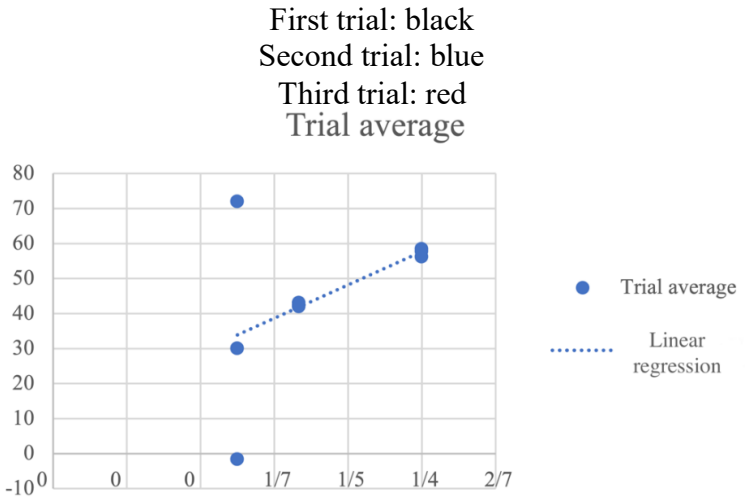


Figure 3: Trend of average zeta potentials shown by a linear regression curve on a scatter chart, each plot representing the average value of one trial
(horizontal axis: mass of DOTAP/mass of PLGA; vertical axis: zeta-potential/mV)

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