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# Development of a DNA-liposome Complex for Gene Delivery Applications

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## Keywords
Cationic liposome, DNA, Transfection, Gene delivery

## Highlights
• Liposomal formulation in this research had a better function than Lipofectamine® 2000.

• The average particle size had no significant change while the PDI increased after lyophilization.

• LacZ expression of the developed cationic liposomes is approximately equal to the Lipofectamine® 2000.

## Abstract

The association structures formed by cationic liposomes and DNA (Deoxyribonucleic acid)-liposome have been effectively utilized as gene carriers in transfection assays. In this research study, cationic liposomes were prepared using a modified lipid film hydration method consisting of a lyophilization step for gene delivery applications. The obtained results demonstrated that the mean particle size had no significant change while the polydispersity (PDI) increased after lyophilization. The mean particle size slightly reduced after lyophilization (520 ± 12 nm to 464 ± 25 nm) while the PDI increased after lyophilization (0.094 ± 0.017 to 0.220 ± 0.004). In addition. The mean particle size of vesicles increases when DNA is incorporated to the liposomes (673 ± 27 nm). According to the Scanning Electron Microscopy (SEM) and transmission electron microscopy (TEM) images, the spherical shape of liposomes confirmed their successful preservation and reconstitution from the powder. It was found that liposomal formulation has enhanced transfection considerably compared to the naked DNA as negative control. Finally, liposomal formulation in this research had a better function than Lipofectamine® 2000 as a commercialized product because the cellular activity (cellular protein) was higher in the prepared lipoplex than Lipofectamine® 2000.

# 1. Introduction

The evolution of genetic engineering and gene therapy is dependent on reliable and productive systems for the incorporation of DNA into the objective cells. The potential use of cationic liposomes in transfection has been reported in the literature [1], [2], [3], [4], [5]. Common agents for this method are cationic liposomes and the complexes (lipoplexes) that they form with, negatively charged natural and synthetic oligo-deoxy-nucleotides (ODN) [6], [7], [8].

A liposome is a spherical vesicle having at least one lipid bilayer. Liposomes can be filled with drugs and genes, and they can be utilized to deliver drugs/gene for cancer and other diseases. Lipoplex is a lipid/liposome and DNA complex that is utilized to deliver genes [9].

The positive charge on liposomes created with cationic lipids ensures binding to cell membranes due to their negative charge [10]. Lipoplexes then enter the objective cells primarily by absorptive endocytosis [11]. Therefore, liposomes are able to act as vectors able to convey anticancer agents for preferential delivery to distal tumor sites following intravenous injection [12], [13], but some contention exists on the advantage of lipoplexes with regard to viral vectors in gene therapy protocols [14].

The utilization of synthetic cationic surfactants in liposomal form is apparent in about 20% of the current clinical trials in gene therapy [15], [16]. Cationic liposomes spontaneously react with negatively charged ODN, such as single-stranded, plasmid DNA, and double-stranded polynucleotides, resulting in the formation of self-assembled complexes that include negatively charged molecules in the condensation reaction [17].

Many studies have focused on lipoplex microstructure and stability by way of different methods and transfection efficiencies [18], [19], [20].

Once the liposomal structures have been created, the maintenance of the physicochemical characteristics during fabrication can be difficult. Leakage of the encapsulated material as a result of a change in the size, permeability of the membrane, and distribution and stability problems that are indicative of potential hydrolytic and oxidative degradation are the common problems during storage. Techniques are offered to overcome these instability problems, those outlined to minimize the degradation process and those that aid liposomes to resist harsh conditions [21].

The problems identified with the lipid oxidation and hydrolysis amid the shelf-life of the liposomal product could be decreased by the storage of liposomal dispersion in the dry state by freeze-drying (lyophilization) without trading off their physical state or encapsulation capacity [21], [22]. Nevertheless, the freeze-drying process of liposomal systems without suitable stabilizers will cause agglomeration of vesicles and, as an effect, physical instability [21]. To encourage vesicle stability amid the freeze-drying process, cycloprotectants, including saccharides (e.g. sucrose) are used [23], [24], [25].

1,2-Bis(oleoyloxy)-3-(trimethylammonio) propane (DOTAP), was first fabricated by Leventis and Silvius in 1990 [26], [27]. This molecule structure includes a quaternary amine head group coupled to a glycerol backbone with two oleoyl chains. Ester bonds, which are hydrolysable, could make the lipid

biodegradable and decrease the cytotoxicity. It is noticeable that this type of monovalent lipid also displayed no cytotoxic effect on near-confluent cell monolayers, additionally showing the same lipoplex sensitivity at 25%–35% cell confluence. DOTAP is completely protonated at pH 7.4, thus it is possible that more energy is needed to separate the DNA from the lipoplex for successful transfection [11], [27], [28]. Therefore, for higher effectivity of DOTAP in gene delivery, it must be combined with a helper lipid, which seems to be the case for most cationic lipid formulations [27].

Most cationic liposomes have a neutral phospholipid part in addition to the lipid$^+$. 1,2-Dioleoyl-*sn*-glycerol-3-phosphoethanolamine (DOPE) is routinely employed as a helper lipid [29]. Improved DNA translocation and transfection efficiency in the presence of DOPE were confirmed by several researchers [8], [30], [31]. DOPE is also selected due to its ability to decrease the cytotoxicity of cationic liposomes. It is fusogenic and displays a strong destabilizing effect toward lipid bilayers, as reported by Litzinger and Huang [30].

However, there are many clinical trials of gene delivery, based on the use of lipoplexes, without sufficient understanding of all the physicochemical characteristics responsible for their action. According to the explanations, any extra information about these systems will help their complete molecular and/or supramolecular examination.

In this research study, we fabricated the cationic liposomes using a modified lipid film hydration method for gene delivery applications. Also, in this contribution, we have employed zeta potential analyzer, SEM and TEM analyses to reveal the structural features of the complexes formed between liposomes and DNA.

## 2. Experimental procedure

### 2.1. Materials

Cholesterol and sucrose were purchased from Sigma-Aldrich and Alfa Aesar, respectively. 1,2-Di-*O*-octadecenyl-3-trimethylammonium propane (DOTAP) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were provided by Avanti Polar lipids. Beta-galactosidase and BCA assay kits were provided from Promega and Pierce, respectively. Lipofectamine® 2000 was purchased from Invitrogen Thermo Fisher Scientific. Human Embryonic Kidney 293 (HEK-293) cell line was purchased from American Type Culture Collection. Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS) and penicillin-streptomycin solutions were obtained from GIBCO Inc. All of the solvents were of analytical grade without any further purifications.

### 2.2. Preparation of liposomes and lipoplexes

Cationic liposomes were prepared using a modified lipid film hydration technique. Briefly, DOTAP/DOPE/cholesterol with molar ratio of 1:1:2 were dissolved in chloroform (0.5 ml, total concentration of 5 mg/ml). The solvent was then eliminated by heating the solution at 40 °C for 6 h in room pressure followed by utilization of a vacuum for 24 h. Consequently, the dry lipid film was hydrated through 1 ml of 15 mg/ml sucrose aqueous solution and bath-sonicated to achieve

multilamellar vesicles followed by lyophilization (− 52 °C, 2.5 Pa) for 24 h to achieve a preservable white powder. The liposomes were reconstituted via incorporation of water to the achieved powders. Eventually, liposome-DNA complexes i.e. lipoplexes with positive-to-negative (±) charge ratio of 6/1 were prepared by mixing appropriate amounts of DNA (LacZ) and cationic liposomes and incubating for 20 min at ambient temperature. The ratio of positive-to-negative charge was calculated based on DNA phosphate group concentration and cationic amine nitrogen (in the cationic lipid DOTAP). The charge ratio was theoretically calculated as mole ratio of DOTAP (one charge per molecule) to nucleotide residue (average MW 330).

## 2.3. Particle size and surface charge of liposomes and lipoplexes

In order to evaluate vesicle formation, the effect of lyophilization on the vesicles' size as well as the influence of addition of DNA on the reconstituted vesicles, mean particle size and size distribution of liposomes (before and after lyophilization) and lipoplexes were assessed utilizing particle size analyzer (Brookhaven Instruments). Additionally, lipoplex formation was investigated through measurement of surface charge utilizing zeta potential analyzer (Brookhaven Instruments). All the measurements were conducted at ambient temperature employing deionized water as the solvent.

## 2.4. SEM observations in the powder form

The morphology of liposomes in the powder form (after lyophilization and before reconstitution) was examined using Scanning Electron Microscopy (SEM, Hitachi S-4800). To prepare appropriate samples for imaging, dry lipid film was hydrated using 2% phosphotungstic acid as negative stain in combination with 15 mg/ml sucrose solution followed by freeze-drying. Imaging was conducted at the accelerating voltage of 10 kV after gold (Au) coating.

In order to obtain additional information about the liposome-DNA complexation, SEM imaging of lipoplexes was also performed. For this purpose, lyophilized lipoplexes were prepared by accomplishing the liposome-DNA incubation step prior to the freeze-drying step.

## 2.5. TEM observations of reconstituted liposomes

The morphology of liposomes after reconstitution was observed using Transmission Electron Microscopy (TEM, JEOL JEM-2100). After incorporation of the deionized water, a certain amount of vesicle suspension was poured on the TEM grid and negatively stained utilizing 2% phosphotungstic acid solution. Imaging was performed after eliminating extra volume of suspension at the accelerating voltage of 200 kV.

## 2.6. Cell culture and transfection assay

In order to evaluate the transfection efficiency of the fabricated lipoplexes, the reporter gene LacZ (12 kbp, 270 μg/ml in TE buffer) and HEK-293 cell line were used. It is worth mentioning that the advantage of employing HEK-293 cells for expressing recombinant proteins contains an efficient

transfection of plasmid DNAs, faithful translation and processing of proteins [31]. The cells were incubated at 37 °C and 5% $CO_2$ in DMEM with 10% FBS and 1% penicillin/streptomycin. One day prior to transfection, the cells were seeded into 12-well plates with a cell density of $3 \times 10^5$ cells per well. Immediately before the test start, culture medium was replaced with serum-free DMEM. Lipoplexes were directly incorporated to the medium and after 5 h incubation, the culture medium was replaced by media with serum and penicillin/streptomycin. LacZ expression was assessed 48 h after transfection utilizing beta-galactosidase (beta-gal) assay and normalized with cellular protein determined via bicinchoninic acid (BCA) assay (BCA assay is a biochemical assay for determining the total concentration of protein in a solution assay). It is worth mentioning that naked DNA and DNA + Lipofectamine were used as the negative and positive controls in this study, respectively.

### 2.7. Statistical analysis

Data was analyzed by Microsoft Excel 2007 software (Microsoft, Redmond, WA) and reported as mean and standard deviation. A two-tailed Student's *t*-test was used for comparing the results between the groups. Furthermore, *p*-value was calculated for every experiment.

## 3. Results & discussion

Cationic liposomes of DOTAP/DOPE/cholesterol were fabricated via a modified dry lipid film technique. The amphiphilic nature of lipids causes self-assembly in aqueous media and multilamellar vesicles are prepared by means of sonication method. Even though the dry lipid film hydration technique is a straightforward and effective method to fabricate liposomes, chemical and physical instability of vesicles in the aqueous media confines the storage time of the formulation. Oxidative and hydrolytic degradation pathways may restrict the shelf life of liposome dispersions. Additionally, the mean particle size and size distribution of liposome dispersions might alter considerably because of vesicle aggregation and fusion [32]. To solve these issues, lyophilization was utilized in which sucrose acted as the lyoprotectant in order to decrease the vesicle agglomeration and instability amid the procedure. Lipoplexes used for transfection could be prepared immediately before use from the obtained lyophilized powder by accomplishing two simple steps: 1 - addition of water to reconstitute the liposomes; 2 - addition of DNA to form liposome-DNA complexes (lipoplexes).

### 3.1. Particle size and surface charge of liposomes and lipoplexes

Table 1 displays average particle size and polydispersity index (PDI) of cationic liposomes before and after lyophilization. Despite the use of bath-sonication, the mean particle size of the obtained liposomes (prior to lyophilization) was relatively large. This could be attributed to the presence of considerable amount of cholesterol in liposome's membrane as the stabilization effect of cholesterol results in a more homogeneous and stable liposome membrane [33]. It has been reported that incorporation of cholesterol in the membrane increases the volume of the inner aqueous liposome core, reduces membrane fusion and consequently donates more homogenous liposomes with bigger sizes compared to pure DOTAP [34].

Table 1. Mean particle size, PDI and surface charge of liposomes (before and after lyophilization) and corresponding lipoplexes.

| Parameter/sample | Liposomes | | Lipoplexes |
|---|---|---|---|
| | Before lyophilization | After lyophilization | |
| **Mean particle size (nm)** | 520 ± 12 | 464 ± 25 | 673 ± 27 |
| **PDI** | 0.094 ± 0.017 | 0.220 ± 0.004 | 0.221 ± 0.024 |
| **Zeta potential (mV)** | 70.2 ± 8.1 | + 73.9 ± 6.6 | + 35.0 ± 8.3 |

The liposomes were lyophilized in order to prolong the storage time. Both the formulation and procedural factors could influence the quality of protection amid lyophilization. In our formulation, both positively charged lipid, DOTAP, and cholesterol could boost protection by boosting stability. The OH moiety of cholesterol can interact with the C $=$O and P $=$O moieties of the lipids (i.e. DOTAP and DOPE) [35], [36]; in addition, cholesterol might debilitate the interaction between the acyl chains and thusly counteract aggregation or boost the interaction between the lipids and lyoprotectant [37], [38]. The lyoprotectant selection, mass proportion of lyoprotectant/lipid and distribution of the lyoprotectant on the two sides of the bilayers are also of considerable significance. Disaccharides such as sucrose used in our formulation have been confirmed to be effective in protecting membrane integrity [39]. Sucrose, as the lyoprotectant in our formulation, is relied upon to secure the vesicles by avoiding fusion, break off bilayers by ice crystals and preserve the integrity of bilayers in the absence of deionized water. Sucrose may form an amorphous, glassy matrix inside and around the liposomes while interacting with lipid headgroups. Furthermore, sucrose could decrease the stress on bilayers led by drying [40].

As it can be observed in the table, the mean particle size slightly decreased after lyophilization while the PDI enhanced after lyophilization. It is expected that inclusion of lyoprotectant i.e. sucrose yields a dried liposome product which, upon rehydration, possessed vesicle size characteristics that approximated to those of the initial liposome formulation, though, the size and PDI following reconstitution was different from the initial size. The results that have been reported for the effect of lyophilization on the mean particle size are controversial. While some authors have reported increase in the liposomes' size [41], others have obtained a reduction in the mean particle size of large liposomes after lyophilization [34], [42].

Considering how lyophilization procedure can possibly affect the liposome size may direct us to the origin of such controversial results. It has been suggested that the freezing process of lyophilization can influence the liposome size significantly and the drying phases might have less impact on the integrity of the liposomes than the freezing phase [34], [43], [44]. The freezing can induce many destabilizing stress factors, such as an increase in liposome concentration, which might result in aggregation or fusion of the liposomes, disruption of the liposomal bilayer structure, owing to the ice-liquid interfaces, and phase separation, resulting in the segregation of the liposomes and the stabilizer(s). Vesicles may diffuse far from ice crystals toward the unfrozen fraction; thus, the particles are gathered in the unfrozen fraction and aggregation is encouraged [45]. On the other hand, osmotic extraction of water

from the vesicles in the freezing step can lead to fragmentation of liposomes, i.e., ice formation in the external phase leads to a progressive increase in the sugar concentration of the residual external solution, which, in turn, dehydrates the vesicles [46]. Therefore, some the outcome might be increase or decrease or even no significant change in the mean particle size depending on which mechanism is dominant while PDI increases in all cases [34], [41], [42], [47]. The decrease we observed in the mean particle size was insignificant ($p$-value > 0.05) while PDI significantly increased ($p$-value < 0.05). It might be a result of concurrence of fragmentation, aggregation and fusion.

The stress factors can be controlled to a large extent by altering process parameters such as the freezing rate, freezing temperature, processing time, type and amount of lyoprotectant [43]. In a study by Vincourt et al., the influence of lyoprotectant/lipid ratio on the particle size of SPC (soybean phosphatidylcholine)/cholesterol/DOTAP liposomal formulations was evaluated; interestingly, the mean particle size increased, remained constant or reduced after lyophilization when a low (3:1), moderate (5:1) or high (10:1) ratio of sugar/lipid was used, respectively [48].

Table 1 also shows average particle size, PDI and zeta potential of lipoplexes compared to the corresponding liposomes. As it can be observed in the table, the average particle size of vesicles increases when DNA is incorporated to the liposomes, which is in agreement with the results published by other researchers [49], [50], [51]. Radwan Almofti et al. revealed that DNA-induced liposome-liposome fusion is responsible for the change in liposome size upon incorporation of DNA [49]. The positive charge of liposomes led by amine groups of cationic lipid, DOTAP, was reduced but remained positive via incorporation of DNA, which supports the formation of liposome-DNA complexes. The net positive charge of the formulations makes them suitable for transfection.

The spontaneous formation of liposome-DNA complexes that are effective in DNA transfection offers that a single plasmid interacts with adequate cationic lipid to completely neutralize the negative charge of the DNA and establish a complex with a net positive charge that can associate with the negatively charged surface of the cell [52].

## 3.2. Morphology of liposomes and lipoplexes in powder form

Scanning Electron Microscopy was used for two purposes: (i) observing the morphology of dry vesicles in lyophilized powder and (ii) investigating the effect of complexation with DNA on the morphology of the vesicles. On the other hand, a scanning electron microscope is extensively employed to detect the microstructure of objects at a resolution of nano to micrometer with its advantage of simple and high throughput handling. To obtain clear micrographs, SEM commonly needs a highly vacuumed condition, which causes liposomes to burst because of evaporation of the aqueous phase. Thus, the imaging of it is quite difficult and we have successfully examined the samples with SEM methods.

Fig. 1 shows the (a) liposomes and (b) lipoplexes morphology in dried form.

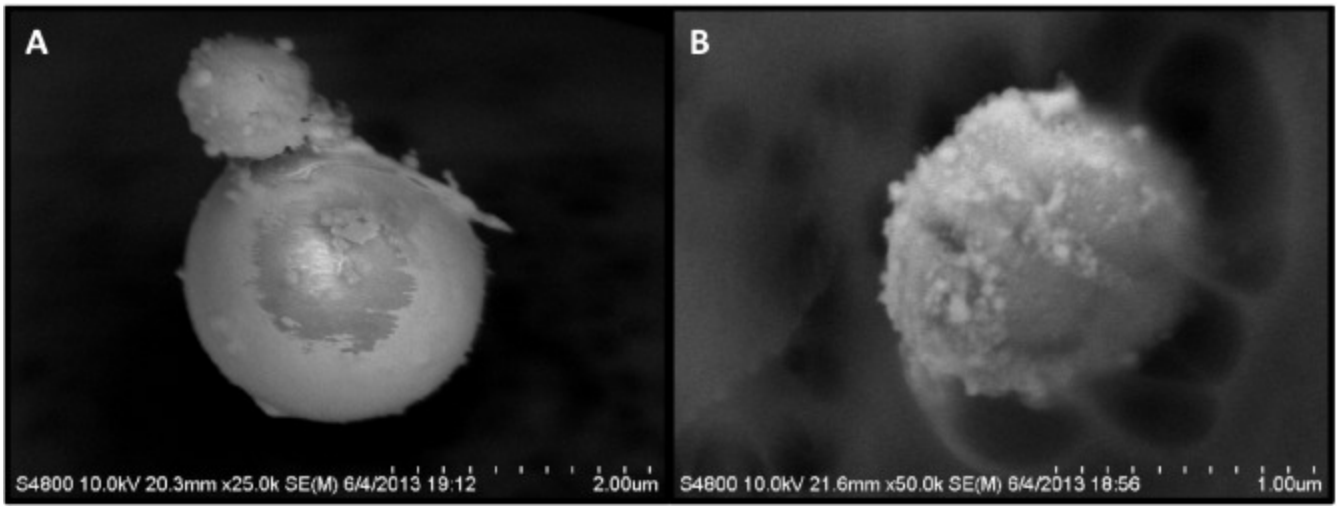


Fig. 1. (a) Liposomes (magnification = 25 k) and (b) lipoplexes morphology in dried form (magnification: 50 k).

There are several models of the microstructure of cationic lipid-DNA complexes in the literature [53], [54], [55], [56], [57]. In general, these models are divided into two categories based on the relative orientations of the cationic lipid and DNA. In one model, as reported by Felgner et al. [58], the DNA adsorbs electrostatically to the outside layer of the cationic vesicles. In another model, the cationic lipid coats the DNA in a lipid shell [59], [60], [61]. The mixtures of the aforementioned two basic models are definitely possible. Nevertheless, we will limit our interpretation of the obtained data to these two models, termed external/outside or internal/inside, as they allude to the position of the DNA relative to the cationic lipid.

Also, it is worth mentioning that the presence of cholesterol applies a significant impact on the characteristics of the lipid bilayers of the liposomes. The addition of cholesterol to a fluid phase bilayer (mainly unsaturated lipids) reduces its permeability to water. Cholesterol molecules fill in the free space that forms because of the kink in the chain of the unsaturated lipids, and this will reduce the flexibility of the surrounding lipid chains. This interaction also enhances the mechanical rigidity of fluid bilayers and reduces their lateral diffusion.

According to these explanations and Fig.1 (increasing the roughness of the surface in Fig. 1b rather than Fig. 1a), we can conclude that our obtained data could be interpreted by external/outside model. Presence of high amount of cholesterol in the formulation may result in the formation of rigid vesicles that only allows DNA condensation on the surface.

3.3. Morphology of liposomes after reconstitution

The reconstitution of vesicles takes place via incorporation of deionized water to the powder. Water diffusion toward the center of vesicles may be prompted by osmotic pressure created by sucrose encapsulated inside the vesicles. Morphology of reconstituted liposomes could be seen via TEM micrographs as displayed in Fig. 2.

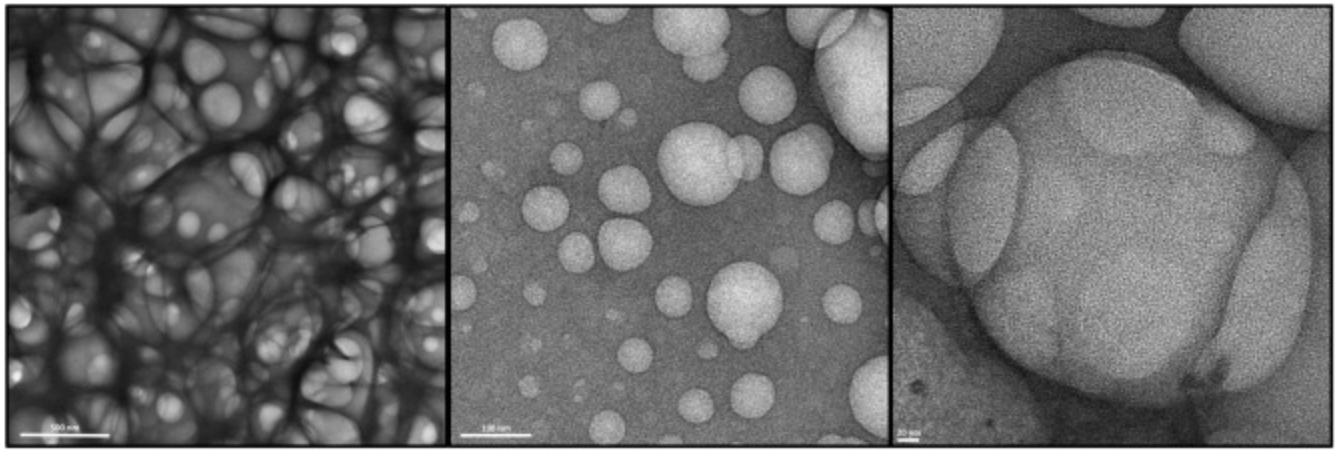

Fig. 2. TEM images of multilamellar vesicles after reconstitution with different magnification (magnification from left to right: 10 k, 40 k and 60 k).

The spherical shape of liposomes affirms reconstitution of them from the powder. In this figure, the vesicles with different sizes were observed due to polydisperse particle size distribution of the samples.

The use of electron microscopy is particularly valuable where proteins or DNA have been encapsulated. Alfredsson reported the use of TEM for research containing DNA and lipid structures (lipoplexes) [62]. For many reasons TEM does not allow a quantitative determination of the limiting DNA/lipid ratio for total entrapment or binding of DNA.

## 3.4. Transfection efficiency

Sample efficiency in transfection was evaluated by means of HEK-293 cells. Naked DNA was employed as negative control while Lipofectamine/DNA complex was selected as positive control in this transfection experiment [63]. The obtained results have been displayed in Fig. 3.

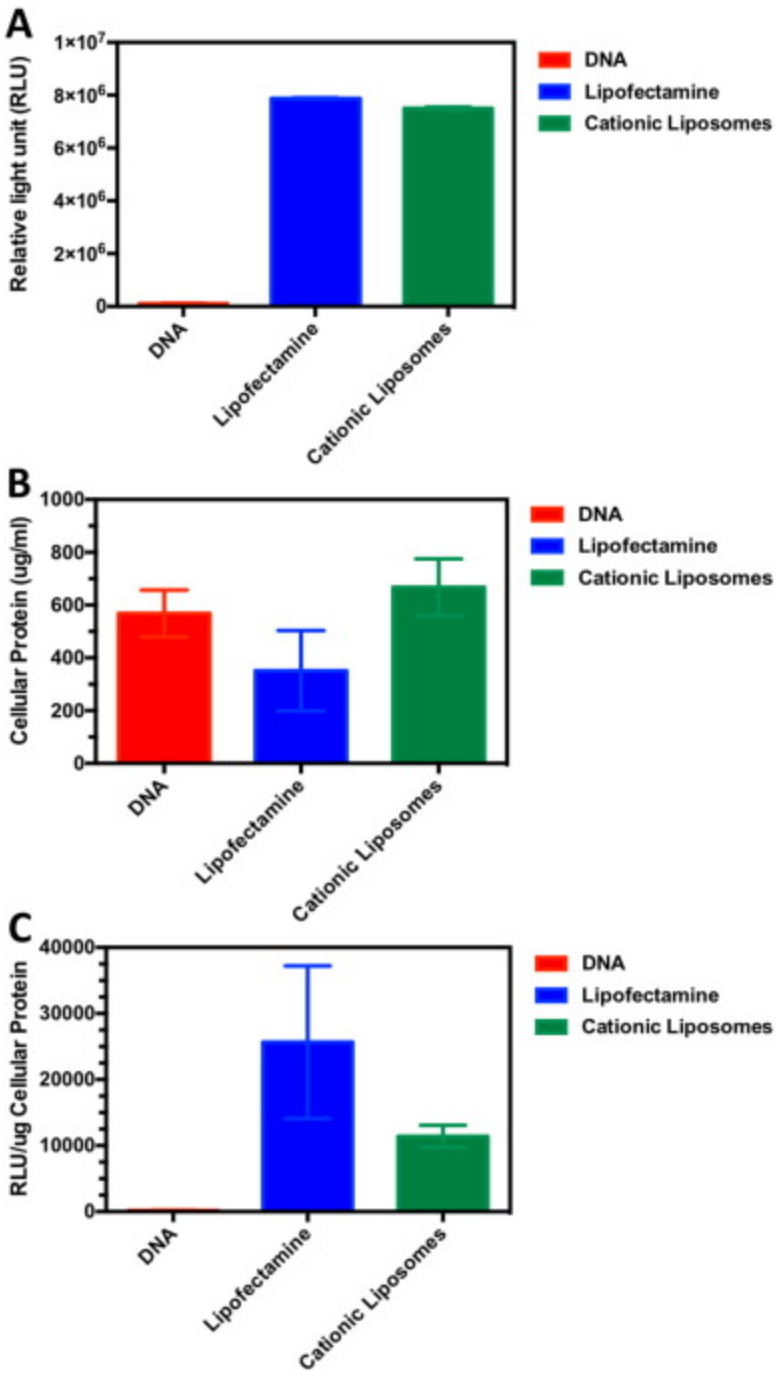


Fig. 3. Transfection efficiency of liposomal formulations (a) LacZ expression, (b) quantification of cellular protein, and (c) LacZ expression per mass unit of cellular protein.

When a transient or stable transfection assay is created for a promoter, a primary objective is to determine promoter strength. Because transfection efficiency in such assays can be low, promoters are commonly fused to heterologous reporter genes that encode enzymes that can be measured utilizing highly sensitive assays. The reporter protein's activity or fluorescence within a transfected cell population roughly corresponds to the steady-state mRNA level. Although the *E. coli* LacZ gene,

encoding beta-gal, can be employed as a standard reporter for monitoring the strength of a promoter or enhancer in a transient or stable transfection assay, it is predominantly utilized as an internal control during transient transfection experiments. When used in this manner, cells are usually transfected with the control plasmid and an experimental plasmid containing reporter gene under the control of the promoter or enhancer of interest [64].

As it can be observed in Fig. 3a, the LacZ expression conducted by the prepared cationic liposomes is almost equal to that of Lipofectamine® 2000. Meanwhile, the cellular protein of the prepared cationic liposomes is higher than Lipofectamine® 2000 (see Fig. 3b). It is worth mentioning that transfection efficiency depends on both formulation and environmental factors and the prepared liposomal formulation had a better function than Lipofectamine® 2000 under the mentioned circumstances.

It is obvious that liposomal formulation has enhanced the transfection dramatically compared to the negative control. Enhanced transfection of different cationic liposome-DNA complexes and its mechanism has been reported by various authors [50], [65], [66], [67], [68], [69], [70], [71]. The improved transfection could be related to the net positive charge of vesicles which lead to high efficiency of DNA complexation, electrostatic interaction with negatively charged cell membrane resulting in adsorptive endocytosis or fusion of the complex with the plasma membrane [52] and increased endosomal escape [65] due to incorporation of helper lipids i.e. DOPE and cholesterol in the formulation. It has been confirmed that physicochemical characteristics of the lipoplexes could impact their mode of cellular uptake [49], [72]. Even though Bennett et al. recommended the endocytic uptake of cholesterol-containing liposomes because of their rigidity [73], an examination by Pozzi et al. cleared that cholesterol-containing lipoplexes enter the cells partially by membrane fusion, and this mechanism represents for effective endosomal escape [33].

Dramatic reduction of cellular protein in the Lipofectamine treated group reflecting cell death might be a result of addition of antibiotics to the media after 5 h period of transfection. It has been reported that DNAs delivered by Lipofectamine® 2000 reach the nucleus with a high frequency only after 4 h incubation [74] and complexes may be removed after 4–6 h according to manufacturer's instructions even though they cause no significant cytotoxicity if remain in the media. However, transfection may continue by means of adsorbed complexes on the surface even after removing the complexes. Permeability of the cells during transfection might results in antibiotic diffusion and toxicity. Decrease in the number of viable cells in turn prevents Lipofectamine from boosting the transfection. On the other hand, our formulation represented no cytotoxicity in the presence of antibiotics. Such an observation may originate from the difference between intracellular trafficking mechanism of Lipofectamine and that of the prepared lipoplex. According to a recent study by Cardarelli et al., Lipofectamine, contrary to DOTAP-based carriers, is able to efficiently avoid active intracellular transport along cytoskeleton components. They found that Lipofectamine becomes inert with respect to the binding machinery that is committed to active transport along microtubules. This ends up into random diffusion within the intracellular environment [75]. Therefore, enhanced permeability of cell membrane during transfection might facilitate non-selective diffusion of other media components like antibiotics.

Finally, it is important to point that cholesterol could boost transfection by protecting the DNA from degradation by DNAses in the body [33]. Additionally, cholesterol has been displayed to be effective in decreasing the binding of serum proteins to lipoplexes, and as a result, boost the transfection of DNA, both in vitro and in vivo [76].

## 4. Conclusion

In conclusion, cationic liposomes of DOTAP/DOPE/cholesterol were successfully fabricated via a modified dry lipid film technique. The results cleared that the average particle size had no significant change after lyophilization. Also, the average particle size slightly reduced after lyophilization while the PDI increased after lyophilization. Furthermore, the average particle size of vesicles enhances when DNA is integrated to the liposomes. With regard to the SEM and TEM observations, the obtained spherical shape of liposomes affirmed their reconstitution from the powder. Finally, the obtained experimental results demonstrated that the LacZ (DNA) expression of the developed cationic liposomes is approximately equal to the Lipofectamine® 2000. Additionally, the obtained cellular protein of the mentioned fabricated cationic liposomes (667.91 µg/ml) is higher than Lipofectamine® 2000 (351.25 µg/ml) as a commercialized product.

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