ABSTRACT
Gene delivery is a process by which an exogenous copy of the gene or DNA is introduced into the cell. Gene delivery has tremendous medical implications in treating several genetic disorders, which are refractory to the conventional medicines. Several strategies have been developed to introduce the foreign DNA into the cells. Among the most efficient ones are viruses, which due to their natural ability to carry the genetic material into the cells have attracted scientists to use them to carry DNA fragment of our interest into the cells. However, due to their harmful potential as well as safety concerns, other alternative non-viral methods have been developed and tested in the recent years to deliver DNA much more safely. One promising vehicle among such non-viral categories is cationic liposomes. Cationic lipids have gained popularity because of the several advantages that they have shown over viral based methods.

Chapter 1
This chapter gives an overview of the gene delivery process and various kinds of gene delivery methods. In order to treat genetic disorders one must be able to provide functional copy of the gene whose function has been lost due to mutations in the chromosomal copy. ‘Gene therapy’ is a broad term used to describe this process. Amongst the existing strategies for delivering DNA molecule into the cells, viruses are the most efficient ones. Viruses however, are unsafe for the following reasons - they are highly immunogenic, the size of the DNA fragment that can be delivered is limited, there is always a possibility of generation of undesirable recombinant viruses and there is little control over their chromosomal integration site. Because of these reasons non-viral mediated gene delivery is gaining importance. Cationic lipids are the most popular gene delivery vehicles amongst the non-viral category. They spontaneously form complexes with DNA and are competent in gene transfer. Several clinical trials have been carried out using cationic lipids as DNA carriers. This chapter also discusses various aspects of cationic lipid: DNA complex (CLDC) and mechanisms of cationic lipid mediated gene delivery both in vitro and in vivo.
Chapter 2

CLDC have been characterised by various biochemical and biophysical methods to understand the physical basis of transfection. This chapter describes the effect of cationic liposomes, on the transcription of DNA templates in vitro. Transcriptional activity of DNA-dependent RNA polymerase at DNA templates complexed with the cationic lipid, varied as a function of N/P charge ratio. At low N/P charge ratios of 0.3:1 and up to 1:1, we observed stimulation in transcription, while at higher N/P charge ratios of 3:1, complete inhibition in the activity occurred. Cetyl tri-methyl ammonium bromide (CTAB), a cationic detergent and polyethylenimine (PEI), a cationic polymer, also brought about similar changes although to a lesser extent. The stimulation in transcription, motivated us to probe into the molecular nature of the lipid/DNA interactions by absorbance spectroscopy and circular dichroism. We observed that upon interaction with lipids, hyperchromicity and susceptibility to micrococcal nuclease of the complexed DNA increased, suggesting that the DNA was partially denatured. Circular dichroism spectroscopy showed that on complexation of DNA with the cationic lipid - 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), the positive ellipticity at 273 nm decreased, accompanied with a red shift, with increasing N/P charge ratio. Thus, results from spectroscopic and enzyme assays suggest that at low N/P charge ratios DNA may be partially unwound.

Chapter 3

This chapter describes a method to study the presence of single stranded DNA in CLDC using potassium permanganate (KMnO₄) as a probe. Cationic lipids and cationic polymers are widely used in gene delivery. Using the cationic lipid DOTAP, we have investigated the conformation of the DNA in DOTAP: DNA complexes by probing with KMnO₄. We observe that the DNA in the CLDC shows presence of single stranded regions. We obtained similar results using other water-soluble cationic ligands such as polylysine and protamine sulphate. Small cations such as spermine and spermidine and the cationic detergent - CTAB, also rendered the DNA susceptible to modification by KMnO₄. Interestingly,
thymidines followed by a purine showed higher susceptibility to cationic ligand mediated melting. The data presented here provides direct proof for melting of DNA upon interaction with cationic lipids. Structural changes subsequent to binding of cationic lipids/ligands to DNA may lead to instability and formation of DNA bubbles in double stranded DNA.

Chapter 4
In this chapter we have investigated the relationship between endocytic activity of a cell-type and its transfection efficiency. We used eleven different cell-types, which represent different tissue types and exhibit large variation in their cationic lipid mediated transfection efficiency. The endocytic activity showed a strong positive correlation (r = 0.85) with transfection efficiency. Treating different cell-types with wortmannin, a PI 3-kinase inhibitor, indicated that depending on the cell-type, CLDC enter cells by either PI 3-kinase dependent or independent pathways. Strong dependency of transfection efficiency on the endocytic activity was further confirmed by comparing the CLDC uptake and transfection efficiency between interphase cells and mitotic cells. In mitotic cells, which have reduced endocytic activity compared to interphase cells, CLDC uptake was inhibited and transfection efficiency was dramatically reduced. The results obtained clearly demonstrate that the uptake of CLDC by endocytosis is the first major molecular barrier in cationic lipid mediated gene delivery.

Chapter 5
Presence of co-lipids or helper lipids enhances the transfection efficiency mediated by cationic lipids. Biophysical studies on CLDC with different co-lipids indicate structural differences but the cell biological basis for their differences in transfection efficiencies are not clearly understood. This chapter deals with the effect of co-lipids on the overall process of transfection. Two popularly studied cationic lipids; DOTAP and dimethyldioctadecylammonium bromide (DDAB) were used along with either L-α-dioleoyl phosphatidylethanolamine (DOPE) or cholesterol as co-lipids to investigate the role of co-lipid in transfection. DOPE
always showed higher transfection efficiency than cholesterol with both the cationic lipids. The relative extent of DNA binding of these four cationic liposome formulations or the relative stability of CLDC did not correlate with their transfection efficiencies. Assessment of internalised CLDC by confocal microscopy revealed that cytoplasmic amounts of CLDC containing cholesterol with DOTAP and DDAB were less in comparison to corresponding CLDC containing DOPE. Interestingly, transmission electron microscopy images of these CLDC suggested that the CLDC containing DOPE formed highly compact and discrete structures, whereas, CLDC particles were connected to one another to form large network-like structures in the case CLDC containing cholesterol. To further elucidate the mechanism of CLDC entry into the cells, different conditions were used to block endocytosis, such as plasma membrane cholesterol depletion, cytochalasin D and wortmannin treatments. Data obtained from these experiments suggest that CLDC containing DOPE and cholesterol show distinct routes of cell entry. From the above data, we propose that the lower efficiencies in transfection by DOTAP: Chol and DDAB: Chol compared with their DOPE counterparts is due to both their inefficiency to enter cells and different endocytic routes of cell entry.

Chapter 6
This chapter investigates the role of Simian Virus 40 DNA sequence composed of promoter-enhancer-origin of replication in gene expression after cationic lipid mediated DNA delivery. One of the steps that limit transfection efficiency of plasmid DNA in non-viral gene delivery, once it is delivered to the cytoplasm is inefficient nuclear import. Recently a region of Simian Virus 40 (SV40), more specifically the 372bp fragment of SV40 genomic DNA encompassing SV40 promoter-enhancer-origin of replication (SV40 DTS), has been shown to enable nuclear import of plasmids carrying these sequences by in situ hybridisations in few cell-types. In this chapter we have addressed the issue of effectivity of SV40 DTS in cationic lipid mediated gene delivery to improve the efficiency of the transfection process by transient reporter gene expression assays in various cell-
types. The gene expression from the plasmid constructs carrying SV40 DTS varied with the cell-type and the plasmid construct used. Such cell-type and plasmid construct dependency on gene expression from plasmid containing SV40 DTS suggests that the gene expression from plasmid is not entirely dependent on the process of nuclear import of plasmid alone but, in addition, is possibly determined by post-nuclear import processes viz. transcriptional regulation.