Inclusion of Cationic and Anionic Lipids

DOTAP is a cationic lipid that is often utilized in liposome formulations, especially those which are intended to deliver nucleic acid therapeutics. The effect of the incorporation of DOTAP into a liposomal formulation on the rate of hydrolysis was evaluated by comparing DOTAP–DOPE (18:0, 18:0) liposomes with liposomes composed of the zwitterionic/neutral lipids, DPPC–DOPE (16:0, 18:1), at pH 7.0 [246]. An increased rate of hydrolysis was observed for both lipids in the DOTAP–DOPE formulation. The Increased rate of hydrolysis for liposomes containing DOTAP was attributed to increased accessibility of ester linkage between the glycerol backbone and the fatty acid tails, thereby promoting hydroxyl-catalyzed hydrolysis [246]. The effect of the incorporation of an anionic lipid, egg phosphatidylglycerol (EPG), into a PHEPC liposomal formulation has also been investigated. An increased rate of hydrolysis was observed for EPG–PHEPC (10:4) liposomes relative to pure PHEPC liposomes, particularly in the pH range of 4–6.5 [65]. The increased hydrolysis rate for EPG–PHEPC liposomes was attributed to an accumulation of protons at the lipid bilayer–water interface leading to increased acid-catalyzed hydrolysis [65]. To our knowledge, there have been no studies which have examined the hydrolytic degradation of lyophilized liposomal formulations. Admittedly, such a study would be very difficult to perform at the low-water contents (~1 %) typical of lyophilized formulations.

Nonviral Vectors as Dehydrated Medicines: Lipid/DNA Complexes

A wide variety of nonviral gene delivery (lipid-based) systems has been developed and continually being improved for *in vitro* and *in vivo* gene delivery (e.g., DNA, siRNA) as a viable alternative to viruses [41, 43, 249–252]. However, it is recognized that these therapeutic systems have been hampered by critical pharmaceutical issues, such as physical and chemical instability, that need to be addressed before nonviral vectors can become a pharmaceutical reality. Due to their rapid deterioration (i.e., aggregation) the prolonged storage of lipoplexes in aqueous formulation is difficult to achieve considering the high sensitivity of DNA to hydrolytic and oxidative degradation [61–63]. Furthermore, as mentioned earlier, agitation is a stress that can occur during shipping and presents crucial difficulties [55, 69, 72]. Since lipoplex-based pharmaceuticals could benefit from ambient storage, lyophilization is a feasible approach to prepare dehydrated formulations of nonviral gene vectors. To date, various additives including trehalose and other sugars have been investigated in an effort to stabilize lipid/DNA complexes during lyophilization, but room temperature stability for pharmaceutically relevant timescales (i.e., 2 years) has yet to be demonstrated. Knowing the high tendency of lipid-based particles to aggregate, appropriate quantities of virtually any excipient should offer protection during freeze-drying [72, 83, 88]. In fact, it has been suggested that the volume