Recent reports have shown that some advanced formulations prepared by these alternative methods have been applied with significant success for *in vivo* studies of therapeutic siRNA [249, 259, 270]. Despite this great progress, prolonged storage of lipoplexes in aqueous formulation yet is difficult to achieve considering the high sensitivity of both DNA and lipid components to hydrolytic and oxidative degradation [61, 66, 67, 79, 271–273]. Furthermore, it has been reported that DNA functionality and structure may be damaged directly by co-oxidation with lipids [274–276]. In the last decade, there was a growing consensus that lyophilization technology is a suitable approach to stabilize batches of nonviral gene delivery systems as dehydrated formulations. However, lyophilization studies have mostly focused on the stability of nonviral vectors during acute freeze-drying stress [83, 85, 89, 90, 95, 99, 116, 117, 120, 185, 277–280]. Previous work has demonstrated that sugars, especially disaccharides, have the ability to preserve lipid-based nucleic acid formulations during acute freeze-drying [83, 86, 90, 95]. In fact, these authors have suggested that a critical sugar to DNA ratio (e.g., 1000, *w/w*) should be sufficient to preserve particle aggregation and vector physicochemical features in a viscous sugar-based matrix [83, 103].

Stability of Lipid/DNA Complexes

Before lipid-based therapeutics develop into a market reality, preparations will need to be physically and chemically stable when stored as dried solids at pharmaceuticals timescales (i.e., 18–24 months). Despite the benefits of storing lipoplexes as dehydrated formulations, only a limited number of studies have assessed their stability in the dried state during short-term and/or prolonged storage (Table 2). As a number of studies have implicated maintenance of particle size as a critical factor for the recovery of transfection activity [83, 90, 91, 95, 99, 103, 120, 181, 193], our recent results have demonstrated a relationship between retention of particle size and T_g of the glassy excipient phase during prolonged storage [183]. This is clearly illustrated by the fact that lyophilized vectors stored at room temperature in trehalose (high T_g; [178, 183, 276, 279]) show less tendency to aggregate as compared to glucose formulations (lower T_gs; [183]). These findings are consistent with the idea that vectors possessing more restricted mobility are less prone to aggregation [127]. It is important to realize that the viscosity of these lyophilized formulations is too high (even slightly above T_g) to allow aggregation in the dried solid. Therefore, we think that the observed particle size increases in our studies were most likely due to physicochemical changes in lipoplexes that promote aggregation upon rehydration [183]. However, despite the maintenance of particle size in many formulations (see Table 2), our previous reports have shown that progressive vector degradation occurred in spite of the high T_g values and low moisture contents, indicating that mechanisms other than aggregation are responsible for the loss of biological activity during storage [178, 179, 183, 276]. It is important to mention that under the experimental conditions of prolonged storage (e.g., 24 months), dried lipoplexes for-