mulated with glucose also exhibited severe physical degradation (i.e., brown cake) indicative of nonenzymatic glycosylation via the Maillard reaction [183]. However, even nonreducing sugars (sucrose and trehalose) failed to stabilize vector formulations during storage, suggesting that other degradation mechanisms such as oxidation (generation of ROS) are active in the dried cake [178, 179, 183]. Similarly, Kundu and collaborators [281] recently have suggested that their nanosome degradation may be the result of ROS formation-related siRNA oxidation. As a matter of fact, fundamental issues associated with the role of ROS in the chemical stability of lipid/DNA complexes in the dried state have been recently elucidated by our group, and data consistently showed that ROS are generated in the dried cake. [115, 179, 183, 185]. We have speculated that trace amounts of transition metals in excipients can catalyze the generation of ROS, and that the high free volume of glasses might facilitate their diffusion [115, 183]. Furthermore, we have determined that the lipid component contributes substantially to the observed oxidative damage in dried cationic lipid/DNA complexes[179]. We have also suggested that the close proximity of DNA to the lipid component in lipoplex formulations facilitates the interaction of DNA with oxidized lipids (e.g., peroxyl radicals) or with other by-products of lipid peroxidation, thereby compromising stability. In fact, our group has observed that TBARS: lipid peroxidation end products or aldehydes [282, 283]) are formed and accumulated in lyophilized cakes during storage [179]. Considering that unsaturated lipids constitute the cornerstone of lipid-based therapeutics [45, 284] and noting that oxidation of lipids via a free radical mechanism is exacerbated in lipids possessing higher degree of unsaturation [235, 285], our group also explored different strategies to minimize oxidative damage in the dried solid during storage [178, 179]. We found that in spite of the inclusion of demetalation and headspace oxygen displacement steps, formation of ROS was still observed in dried formulations, which suggests that these approaches are not sufficient to protect lipoplexes. However, the inclusion of a metal ion chelator (DTPA, 200 μM) or a lipid-soluble antioxidant (α-tocopherol, 50 μM) to our formulations prior to lyophilization enhanced the stability of dried lipoplexes during storage [178]; formulation strategies that have been applied in subsequent lipid-related storage studies [276].

Effect of Moisture on Lipid/DNA Complex Stability

The effect of moisture content on the chemical and biological stability of lipid/DNA complexes during storage has been recently explored by our group and others [178, 276, 279]. Considering that water is a potential source of ROS [191, 286] and that it can participate in hydrolytic reactions that can chemically degrade biomolecules during storage [138, 229], it is typically assumed that lower water contents might enhance the stability of lipoplexes in the dried state. To this respect, recently, Molina and Anchordoquy [178] showed that oxidative damage was considerably enhanced in lyophilized preparations possessing low T_gs (~56 °C) and relatively higher moisture content (~2%) after 2 months of storage at higher temperatures (i.e., 40 °C, 60 °C), consistent with previous studies that suggest that maximal sta-