with sugar excipients such as sucrose or trehalose added not only to stabilize the formulation during the freeze-drying process but also to achieve isotonicity after rehydration (e.g., 10% sucrose). While current pharmaceutical preparations for clinical use are more concentrated (e.g., ≥0.5 mg plasmid DNA/mL), formulate at high excipient to DNA ratios will make it more challenging. It is also recognized that high concentrations of sugars lengthen the lyophilization cycle, resulting in considerable production costs [56, 102]. Therefore, the development of more stable compositions at lower excipient to DNA ratios is highly desirable. For example, the group of Quaak and collaborators [122] has been able to manufacture a GMP-lyophilized dosage form at a low sugar to DNA ratio of 20% (*w/w*).

Stability of Dried Plasmid DNA

Despite the wide interest for the need of preserving DNA in the dried state, there are limited studies that provide stability data during and/or after lyophilization of naked DNA as pharmaceuticals. With regard to the stability of naked DNA during the lyophilization process, it has been reported that naked DNA undergoes double-strand denaturation in the absence of lyoprotectants [91], an observation that is consistent with previous findings where DNA does not retain its native structure (i.e., double helix) and is more vulnerable to oxidative damage when stored under extremely dry conditions (e.g., in the presence of phosphorus pentoxide) [138]. However, DNA must resist the freezing and drying stresses encountered during processing before stability can be addressed. In this respect, it has been demonstrated that the addition of disaccharides, such as sucrose and trehalose, are beneficial to prevent loss of SC DNA during lyophilization [55, 56, 82, 91, 96, 113, 115, 121, 178, 179].

Prolonged stability of plasmid DNA as dried formulations is crucial for their development as viable pharmaceutical products. The effects of prolonged storage (18–24 months) of dried DNA were first reported by Kolobov and Vainberg almost 40 years ago [180]. Their initial findings demonstrated that dry CT-DNA samples, in the absence of lyoprotectants and stored under refrigerated conditions, experienced a progressive strand breakage (~35% loss in molecular weight) over a period of 14 months. However, biological activity was not measured. More than 20 years later, a preliminary accelerated study by Cherng and collaborators have suggested that dried naked plasmid DNA containing high amounts of sucrose (~2500 sugar to DNA weight ratio) could be stored at room temperature and slightly higher temperatures (40 °C) for up to 10 months [181]. Yet, despite the increasing interest in preparing dry plasmid DNA formulations for pharmaceutical purposes, only a limited number of studies have reported the stability of plasmid DNA during storage (as shown in Table 1). One report, for example, showed that biological activity of lyophilized sugar-containing naked DNA preparations could be fully maintained for up to 3 weeks at high temperatures (i.e., 75 °C). However, formulations were stored at very high sugar to DNA ratios (i.e., 4000, *w/w*) [91]. In contrast, a later study has reported that purified plasmid DNA prepared at a ratio of sugar to DNA much lower (i.e., 34.2 *w/w*) and dehydrated under extreme conditions (on the surface of phosphorus pentoxide powder, P_2O_{50}) at room temperature underwent oxidative damage after 56 days even in