during acute lyophilization [115] and during storage [110, 178]. DTPA-prevented ROS levels increase in the presence of Fe^{2+} (400 μM), which is consistent with the fact that its affinity for iron (log K_a of Fe^{2+} = 16, [189]) is larger than the affinity of iron for double-stranded DNA (log K_a of Fe^{2+} = 4.73, [190]). Similar to that discussed below for lipid oxidation (see "Stability of Lipid/DNA Complexes" section).

Although vitrification is not required for vector stability during acute freezing or dehydration [83, 102], minimizing biomolecule (e.g., DNA) mobility via storage well below the glass transition (T_g) of the dried product might be necessary for long-term stability. Recently, our group has dried DNA in the presence of sugar combinations (Fig. 2a, [110]), showing that some formulations are better than others during acute lyophilization. We observed that 5 % trehalose and 2.5 % sucrose/2.5 % hydroxyethyl starch (HES)-based formulations seem to maintain most of the initial SC content in the presence of DTPA. Compared to fresh samples, formulations containing higher amounts of HES did not work very well and dropped down to approximately 80 % (e.g., pure HES, 4 %HES/1 % trehalose or 4 % HES/1 % sucrose). It is important to point out that this loss in SC content during acute lyophilization is unacceptably high for a pharmaceutical product. Furthermore, we have observed that trehalose-containing formulations maintain DNA integrity for 9 weeks at higher temperatures (e.g., 40, 50, and 60 °C) in the presence of DTPA, and also resulted in better preservation of the initial biological activity (Figs. 2b, 3a, b, [110]). In our hands, however, pure sucrose-containing formulations did not protect naked plasmid DNA in terms of biological activity and DNA integrity even at 40 °C (Figs. 2b, 3d). Although the addition of HES into sucrose-containing formulations (e.g., 2.5 % HES/2.5 % sucrose) increased biological activity during storage, it is clear that adding more HES to the formulation diminishes the protective effect (compare Fig. 2d–f). Similarly, when plasmid DNA was freeze-dried in HES alone and stored under accelerated conditions, our results showed that after 4 weeks of storage the biological activity of formulations is mostly lost (data not shown). Although these data are not sufficiently extensive to permit firm conclusions concerning the role of T_g in prolonging stability of plasmid DNA in the dried state, the observed DNA degradation even in formulations with maximized T_g values (e.g., HES) suggests that glass formation does not correlate with DNA stability which is consistent with our previous reports [110, 183, 185]. Supplementary strategies need to be developed in addition to chelator(s) incorporation in dried DNA formulations in order to reduce product degradation during storage.

Moisture Effect on DNA Preservation

Considering that nucleic acids are highly susceptible to hydrolysis and oxidation in solution [62, 63, 191], one might conclude that the removal of water would increase DNA stability. Although residual moisture (RM) is considered a contributing factor to the degradation of dehydrated protein formulations [192], the role of RM in naked plasmid DNA formulations has yet to be investigated. Research, however, has shown that stability of dried viral vectors is not always maximized at the lowest