



Fig. 1 Sugar maintains dry plasmid DNA (5.9 kb) in its native B-conformation: A gradual anti-symmetric phosphate (PO_2^-) stretching redshift from 1240 to 1227 cm^{-1} is observed upon an increase of trehalose in the formulation from 0 to 5 trehalose to DNA ratio (w/w). Hydrated (*closed square*), dry DNA-containing trehalose (*open circle*) formulation absorptions obtained by Fourier transform infrared (FTIR) spectroscopy. The results are expressed as mean values ± 1 SE of measurements of triplicate samples. w/w , weight to weight ratio, SE , standard error

by Zhu and collaborators [157], who reported that trehalose has a stabilizing effect on the structure of calf-thymus NaDNA (CT-DNA) in the dry state. When lyophilizing nucleic acid-based pharmaceuticals, scientists most likely formulate DNA, as seen in Table 1, in the presence of sugars (e.g., disaccharides) under conditions of low ionic strength (e.g., 2–10 mM salt concentration) and in the presence or absence of a chelator (e.g., diethylene triamine pentaacetic acid, DTPA; or ethylenediaminetetraacetic acid, EDTA) and, generally, dry DNA will be probably in its native B form [157, 158]. However, further studies are needed to conclusively elucidate if the maintenance of the B-like structure in dried DNA formulations is beneficial for pharmaceutical applications (e.g., prolonged storage).

Formulation of Plasmid DNA

Development of a stable and efficacious plasmid DNA formulation (drug product) involves careful consideration of factors (e.g., DNA purity, optimum pH, identification/addition of stabilizers including antioxidants and/or metal ion chelators) that can affect its safety, potency, as well as its chemical and physical stability. Production of high quality of plasmid DNA (i.e., current good manufacturing practice (cGMP)-grade SC of > 3000 base pairs) is an essential prerequisite to ensure that the product specifications are met (purity, potency, identity, efficacy, and sterility). In addition, bulk plasmid purity specifications must meet minimum release values of