of the unfrozen fraction at a critical excipient to DNA ratio is sufficient to isolate lipid/DNA complexes in a viscous sugar matrix, thus preventing aggregation during freeze-drying. An excipient to DNA weight ratio of approximately 1000 was sufficient to achieve complete protection by either sucrose or glucose [83], which is in good agreement with later studies on the stabilization of different types of lipid/DNA complexes [103]. Indeed, a prominent drawback of these potential medicines in a solid phase is their tendency to undergo physical and chemical damage during storage [179, 183], which in turn constitutes a critical pharmaceutical problem for the development of lipoplexes as marketable therapeutic products [56, 58]. These stability challenges are discussed below.

Formulation of Lipid/DNA Complexes

A well-designed liposomal delivery composition will be capable not only of increasing potency of gene-based drugs and decrease the risk associated with toxicity, but also of maintaining its physicochemical characteristics during storage. Certainly, a nucleic acid-containing particle should have a small size (less than 100 nm) and better *in vivo* stability in order to be suitable for systemic gene delivery [253, 254]. Furthermore, formulation factors (i.e., number of components, cation to nucleic acid phosphate charge ratio (+/−), concentration of components, order and mixing rate of the components, ionic strength, as well as temperature of assembly and/or preparation) required to prepare stable particles requires appropriate vector design and a robust method of manufacturing. Considering that most delivery systems incorporate cationic and unsaturated agents (as mentioned above) that interact electrostatically with nucleic acids to facilitate both encapsulation and intracellular delivery [255], various encapsulation technologies have been developed to generate more advanced lipid delivery systems (i.e., multiple lipid component compositions that employ a mixture of cationic lipid, neutral lipid, fusogenic helper lipid, and PEG lipid) needed for therapeutic application. For example, Tekmira Pharmaceuticals (British Columbia, Canada), in partnership with Alnylam Pharmaceuticals (Massachusetts, USA) has developed specialized liposome nanoparticles termed a stable nucleic acid lipid particle (SNALP) that represents the most advanced systemic siRNA delivery system [256–259]. Furthermore, Alnylam Pharmaceuticals has recently reported their positive initial data of phase II open-label extension (OLE) study with patisiran (ALN-TTR02), an RNAi therapeutic targeting transthyretin (TTR) that is currently under development for the treatment of TTR-mediated amyloidosis (http://www.alnylam.com/product-pipeline/ttr-amyloidosis-fap/; [260]). Indeed, lipid-based formulations manufactured at large scale for clinical applications will need to employ pharmaceutically robust methods that are easy to scale-up, cost-effective, and meet regulatory requirements. As a matter of fact, within the past two decades, several methods have been developed to enhance nucleic acid encapsulation in multicomponent liposomal systems including passive (lipid film) technology [261, 262], ethanol dialysis [257, 263], reverse-phase evaporation [264], detergent dialysis [265–267], and, recently, hydration of a freeze-dried matrix [268, 269].