freeze-drying stress. Considering the complexity involved in the stabilization of individual vectors (e.g., naked DNA, lipid) as dehydrated formulations, this chapter addresses these challenges separately. Mechanisms of lipid degradation as liquid and dried formulations are considered. Information highlighting progress to date on the development of dehydrated formulations are reviewed, with particular emphasis on the instability events occurring in the storage of lyophilized naked DNA, unsaturated lipids, and lipid/DNA complexes, and their implications on the development of stable dried pharmaceutical preparations.

Preservation of DNA

The interest in preserving DNA as dried preparations has increased vastly in response to the growing demand from a variety of fields, including pharmaceutical sciences [56, 82, 91, 113, 121, 122], forensics [123], molecular diagnostics [124], and biorepository management [125]) for storing DNA samples or tumor specimen banks [114, 126] for prolonged periods of time, sometimes in large numbers, in regions where sophisticated storage equipment may not be available. Although the requirements are quite different in each of these fields, the need for strategies that provide highly stable storage conditions (e.g., room temperature storage) seems to be a common factor. From a pharmaceutical point of view, while clinical trials are advancing and consequently large amounts of pharmaceutical-grade DNA will be required at the industrial scale, the necessity of developing stable dehydrated DNA formulations, with diminished chemical and/or physical degradation, has gained considerable attention in the recent years. Among the several existing approaches to dehydrate DNA (spray freeze-drying, spray drying, air drying, freeze-drying, or lyophilization), freeze-drying is an established process that can offer a practical alternative to remove water and reduce molecular mobility [127], and, consequently, diminish hydrolytic reactions that are known to damage DNA in solution [76, 128]. Among these technologies, lyophilization has been the preferred approach to remove water due to the general perception that uncomplexed plasmids as well as oligonucleotides may undergo shear-induced damage [129–132] during the process of spray drying. However, recent studies on the mechanisms of nucleic acid shear-induced damage have concluded that smaller plasmids (~5 kb) are not as sensitive to shear stress as larger plasmids (~9.8 and 37 kb) [133, 134] which is consistent with previous findings by Levy and collaborators where the level of damage during shear stress was found to be dependent essentially on plasmid size and ionic strength [135]. These findings are important when considering the practical challenges of producing large amounts of plasmid DNA for therapeutic use in humans, for both clinical trials, and, in the end, full-scale production. In this respect, it is recognized that SC plasmid DNA is frequently subjected to fluid stress during primary isolation processes, and, thus, shear forces involved in upscaling must be controlled [136, 137].