Low Molecular Weight Dextrans Stabilize Nonviral Vectors During Lyophilization at Low Osmolalities: Concentrating Suspensions by Rehydration to Reduced Volumes

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ABSTRACT: Stabilization of nonviral vectors during freezing and drying requires formulation with protective excipients such that transfection rates and physical characteristics are maintained upon reconstitution. While many studies have demonstrated the ability of disaccharides (e.g., sucrose) to effectively protect nonviral vectors during lyophilization, the sucrose/DNA weight ratios required to achieve stability result in formulations that are not osmotically compatible with the subcutaneous (SC) or intramuscular (IM) injection of a typical dose of plasmid DNA. In an effort to reduce the formulation osmolality, dextrans possessing a range of molecular weights were investigated for their ability to serve as protectants. Dextran 3000 proved to be the most effective of the dextrans tested, and offered similar protection to sucrose on a weight basis. However, the advantage of employing this excipient is that the resulting osmolality is reduced by approximately 40% as compared to an equivalent weight of sucrose. Moreover, the use of dextran allows lyophilized vector preparations to be rehydrated to reduced volumes, essentially concentrating vectors prior to administration. Utilizing a combination of dextran 3000 and sucrose, we demonstrate that complexes of polyethylenimine (PEI) and DNA lyophilized at 0.1 mg/mL can be concentrated tenfold upon rehydration, resulting in an isotonic formulation containing 1 mg/mL DNA that can provide more realistic injection volumes for animal studies, and is compatible with clinical trials involving SC and IM injection. J Pharm Sci 94:1226–1236, 2005

Keywords: nonviral vector; gene delivery; lyophilization; freezing; stabilization; formulation; freeze-drying

INTRODUCTION

Problems associated with the use of viruses to deliver genes have stimulated an interest in developing synthetic gene delivery vehicles that do not elicit a specific immune response.[1–6] While much progress has been made in developing nonviral vectors, the delivery efficiency of synthetic systems remains low. As a result, most of the research effort in the field of nonviral gene delivery has focused on designing more efficient vector systems for use in gene therapy. The predominant focus on improving delivery efficiency has led researchers to ignore many pharmaceutical issues that are critical in the development of a commercially viable therapeutic. For example, it is well known that aqueous suspensions of nonviral vectors aggregate over time.[2,7–9] Although early clinical trials prepared vectors at the bedside immediately prior to injection, this approach is clearly not practical for routine clinical administration. Instead, it would be desirable to develop

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dehydrated formulations that are resistant to shipping stresses and could be transported and stored at ambient temperatures.

Recent studies have shown that vector preparations can be frozen or lyophilized in the presence of sugars, but sufficient levels of excipients must be employed to spatially separate particles and prevent aggregation during freezing and drying.[10] In their investigation of this effect, Allison et al.[10] demonstrated that sugar-to-DNA weight ratios of approximately 500–1000 are required to prevent increases in vector particle size and preserve biological activity during the freezing step of a lyophilization cycle. Considering that disaccharides (i.e., sucrose and trehalose) have been identified as the most effective protectants, the sugar-to-DNA weight ratio required for stability corresponds to a DNA concentration of 0.1–0.2 mg/mL in isotonic disaccharide formulations (≈10% sucrose). Although such low DNA concentrations are suitable for transfection studies in cell culture, clinical applications typically require an injectable dose of at least 1 mg of DNA.[11] It follows that at the current levels of disaccharide needed for stability, a 1 mg dose of DNA would need to be diluted in 5–10 mL of isotonic sucrose; a volume too large for applications involving subcutaneous (SC) or intramuscular (IM) injection. Therefore, it would be advantageous to develop vector formulations for injection that are stable at lower tonicities such that more conventional dosage volumes (i.e., ≤2 mL) can be employed in the clinic.

In addition to the problems with tonicity described above, another significant problem with the *in vivo* application of nonviral vectors is the method by which the delivery systems are produced. Current methodology involves the mixing of cationic agents (e.g., polymers, liposomes) with polynucleotides (e.g., plasmid DNA, siRNA) such that electrostatic interactions between the oppositely charged components causes complexation resulting in a colloidal suspension of vectors.[12] Not surprisingly, the mixing conditions play an important role in ultimately determining the physical characteristics of the vectors.[11] While this process is known to result in particulate vectors with heterogeneous characteristics,[13–17] the mechanism of particle formation and mixing makes it very difficult to prepare formulations at the high concentrations necessary for clinical trials employing SC or IM injection, and also for animal studies.[11] This is particularly problematic at +/− charge ratios above neutrality that have been shown to be more efficacious for *in vivo* applications.[18–20] As mentioned above, clinical trials typically employ a DNA dose of at least 1 mg, preferably as an isotonic formulation. For applications utilizing SC or IM injection (e.g., vaccines), this dose must be formulated in a volume that is suitable for injection (i.e., ≤2 mL). These specifications require vectors to be formulated at DNA concentrations of at least 0.5 mg/mL; conditions that foster aggregation and precipitation when vectors are prepared by conventional methods.

Interestingly, it is exactly this problem of low DNA concentration that forces many animal studies to inject unrealistic volumes (e.g., 1 mL containing 100 μg DNA) intraveneously into a mouse possessing a total blood volume of approximately 2 mL. Although the results from such studies were the impetus for many companies to test their vector formulations in the clinic, subsequent work has shown that gene delivery is artificially enhanced under these conditions by a process now known as hydrodynamic delivery.[21] In an effort to employ more realistic transfection conditions in animal studies (e.g., 100 μL injections in mice containing 100 μg DNA), it would be advantageous to develop methods of concentrating vector suspensions to achieve higher DNA concentrations that could also be utilized in clinical trials. Although vector suspensions could potentially be concentrated by utilizing low volumes when rehydrating lyophilized preparations, this approach would further exacerbate the problems associated with the high osmolality of stabilizing disaccharides discussed above.

Considering the problems with hypertonicity due to the high levels of disaccharide needed to maintain vector stability during lyophilization, we investigated the use of polymeric sugars (i.e., dextrans) that would result in lower osmolalities even when employed at the same sugar:DNA *weight* ratio as disaccharides. Our previous work has concluded that high molecular weight polymers do not possess sufficient conformational flexibility to permit effective stabilization,[10,22] consistent with previous work on proteins.[23,24] However, lower molecular weight polymeric sugars (e.g., dextran 1000–25000 Da) could potentially serve as effective stabilizers while reducing the tonicity of lyophilized formulations upon rehydration.[23–25] In this study, we explore the ability of dextrans (molecular weight range 1000–24500) to stabilize nonviral vectors during lyophilization. In addition, we take advantage of the lower tonicities generated by these excipients and assess the potential for vector preparations to be