94°F). This created a logistical challenge for the distribution and storage of the vaccine. Lyophilization is a freeze-drying technique which removes water *via* freezing then sublimation under vacuum. Lyophilizatoin provides long term stability at ambient temperature and at 4 °C. Thus, there are considerable benefits of a lyophilized formulation of the mRNA vaccine formulation. Storage and transportation can be conducted under standard refrigeration and not at extreme levels of freezing temperatures, which would be beneficial to regions of the world without proper storage units or for regions too far from distribution centers. The amount of time the vaccine can be stored will be extended by lyophilization as well. However, there are also obstacles to overcome in arriving at a lyophilized product that has the same *in vivo* same effectiveness as the liquid formulation. Formulation components affect the thermal behavior of the formulation. Complex formulations, for example vaccines that are made up of several antigens or multiple strains, can result in challenging critical formulation temperatures and complicated freeze-drying processes. Freezing and drying can stress a vaccine formulation. For example, in the primary drying step of the lyophilization cycle, drying above the critical formulation temperature results in increased mobility of the amorphous phase which allows protein interactions and can increase membrane permeability.

[0006] Accordingly, there is need in the field of nucleic acid therapeutics (*e.g.*, vaccine therapeutics) for lyophilized formulations that maintain similar properties and effectiveness as the liquid form.

SUMMARY

[0006a] In one aspect, the present disclosure refers to a lyophilized composition comprising a capsid-free closed ended non-viral DNA (ceDNA) vector comprising at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein the at least one nucleic acid sequence encodes a therapeutic protein; and wherein the ceDNA is snap frozen in a sucrose solution prior to lyophilization.

[0006b] In another aspect, the present disclosure refers to a method of expressing a therapeutic protein in a cell comprising contacting the cell with the lyophilized composition as described herein, wherein the cell is in vitro or ex vivo.

[0006c] In another aspect, the present disclosure refers to a use of the lyophilized composition as described herein in the manufacture of a medicament for expressing a therapeutic protein in a cell.

[0006d] In another aspect, the present disclosure refers to a use of the lyophilized composition as described herein in the manufacture of a medicament for treating and/or preventing a bacterial, a viral, a parasitic or a fungal infection in a subject, wherein the medicament is to be administered to the subject.

[0006e] In another aspect, the present disclosure refers to a use of the lyophilized composition as described herein in the manufacture of a medicament for treating and/or preventing a cancer in a subject, wherein the medicament is to be administered to the subject.

[0006f] In another aspect, the present disclosure refers to a use of the lyophilized composition as described herein in the manufacture of a medicament for treating and/or preventing an autoimmune disease or disorder in a subject, wherein the medicament is to be administered to the subject.
[0006g] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the lyophilized composition as described herein.
[0006h] In another aspect, the present disclosure refers to a kit comprising the lyophilized composition as described herein, the pharmaceutical composition as described herein.
[0006i] In another aspect, the present disclosure refers to the lyophilized composition as described herein for use in therapy.

BRIEF DESCRIPTION

[0007] Provided herein are lyophilized compositions comprising a ceDNA vector comprising at least one nucleic acid sequence between flanking inverted terminal (ITRs), wherein the at least one nucleic acid sequence encodes a therapeutic protein.
[0008] There are considerable benefits of a lyophilized ceDNA composition (*e.g.*, a therapeutic composition, *e.g.*, a vaccine composition). For example, storage and transportation can be conducted under standard refrigeration and not at extreme levels of freezing temperatures, for a longer duration of time.
[0009] According to one aspect, the disclosure provides a lyophilized composition comprising a capsid-free closed ended DNA (ceDNA) vector comprising at least one nucleic acid sequence between flanking inverted terminal (ITRs), wherein the at least one nucleic acid sequence encodes a therapeutic protein. According to one embodiment, the therapeutic protein is an antigen, or an immunogenic peptide. According to one embodiment, the antigen, or the immunogenic peptide, is derived from a bacterial, a viral, a fungal or a parasitic infectious agent. According to one embodiment, the antigen, or the immunogenic peptide, is a tumor associated antigen. According to one embodiment, the immunogenic peptide, is associated with an autoimmune condition. According to some embodiments of the above aspects and emboidments, the antigen, or the immunogenic peptide, is selected from one or more of those set forth in Tables 1-7. According to some embodiments of the above aspects and emboidments, the composition is stable at ambient temperature