need. In addition, the development of methods for GMP-compliant manufacturing of injectable RNA lipoplex particle formulations where long shelf-life is provided is still an unmet need.

Thus, there is a need of providing formulations for the delivery of biologically active RNA to a target tissue where the delivered RNA is efficiently translated into the peptide or protein it codes for. Furthermore, there is a need of providing such formulations which are shelf-stable without substantial loss of the product quality and, in particular, without substantial loss of biological activity of the RNA.

The inventors surprisingly found that the RNA lipoplex particle formulations described herein fulfill the above mentioned requirements.

Summary

In one aspect, the disclosure relates to a composition comprising: RNA lipoplex particles comprising: RNA, and at least one cationic lipid and at least one additional lipid, sodium chloride at a concentration of about 10 mM or less, a stabilizer at a concentration of more than about 10% weight by volume percent (% w/v) and less than about 15% weight by volume percent (% w/v), and a buffer.

In another aspect, the disclosure relates to a composition comprising: RNA lipoplex particles comprising: RNA encoding a peptide or protein comprising at least one epitope, DOTMA and DOPE in a molar ratio of about 2:1, wherein the ratio of positive charges to negative charges in the composition is about 1.3:2.0, sodium chloride at a concentration of about 8.2 mM, sucrose at a concentration of about 13% (w/v), HEPES at a concentration of about 5 mM with a pH of about 6.7, and EDTA at a concentration of about 2.5 mM.

In another aspect, the disclosure relates to a liquid composition comprising RNA lipoplex particles obtainable by thawing the frozen composition as defined herein.

In another aspect, the disclosure relates to a liquid composition comprising RNA lipoplex particles obtainable by dissolving the dehydrated composition as defined herein.

In another aspect, the disclosure relates a method of preparing a liquid composition for direct administration to a subject comprising RNA lipoplex particles comprising thawing the frozen composition as defined herein.

In another aspect, the disclosure relates to a method of preparing a liquid composition for direct administration to a subject comprising RNA lipoplex particles comprising dissolving the dehydrated composition as defined herein.

I. Methods for preparing RNA lipoplex particles, RNA lipoplex particles and compositions comprising RNA lipoplex particles

In a first aspect, the present disclosure relates to methods for preparing RNA lipoplex particles with improved biological activity, RNA lipoplex particles prepared according to the disclosure and compositions comprising such RNA lipoplex particles. The RNA lipoplex particles and compositions comprising RNA lipoplex particles are useful for delivery of RNA to a target tissue after parenteral administration, in particular after intravenous administration. The RNA lipoplex particles are prepared using liposomes that are obtained by injecting a highly concentrated solution of the lipids in ethanol into water or a suitable aqueous phase. In one embodiment, the RNA lipoplex product is characterized by a specific pattern in x-ray scattering, where a single Bragg peak at about 1 nm^{-1} is observed, where the peak width is smaller than 0.2 nm^{-1}.

In one embodiment, the liposomes and RNA lipoplex particles comprise 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA) and 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine (DOPE). The concentration of DOPE in the lipid mixture is higher than the equilibrium solubility of the DOPE in ethanol alone. At room temperature, DOPE alone has a solubility of about 50 mM, together with the DOTMA it has a solubility of 100 mM or higher. Lipid solutions to form liposomes, from which highly active lipoplexes are obtained may have a total lipid concentration of 270 mM or above (e.g., DOPE at 90 mM or above). Solutions of even higher concentration in ethanol may be obtained by increasing the temperature. Liposomes obtained from lipid solutions where the concentration of the DOPE is above the equilibrium