METHOD OF MAKING SMALL LIPOSOMES

This application claims the benefit under 35 USC 119(e) and the Paris Convention of U.S. provisional application 61/138,353, filed December 17, 2008, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates generally to the field of liposomal vaccine production.

SUMMARY OF THE INVENTION

In a first aspect, there is provided a composition comprising liposomes of constrained particle size wherein at least about 90% of the liposomes are of a particle size less than about 200 nm and wherein the liposomes further comprise a MUC-1 peptide, or a glycosylated and/or lipidated derivative of a MUC-1 peptide,

wherein the composition is obtained by a process comprising the steps of:

providing a substantially continuously flowing stream of water,

providing a substantially continuously flowing stream of an organic solvent, said organic solvent containing, dissolved therein, at least one lipid and at least one MUC-1 peptide, the lipid or lipids being capable of forming liposomes,

substantially continuously mixing said stream of water and the stream of organic solvent, so as to obtain a mixture, and

cooling the mixture, and

allowing liposomes to form within the mixture,

wherein the ratio of the flow rate of the stream of water to the flow rate of the stream of organic solvent, and the rate of cooling of said mixture, are controlled so as to obtain a preparation of liposomes such that at least about 90% of the liposomes are of a particle size less than about 200 nm.

In a second aspect, there is provided a composition as defined above, wherein the liposomes comprise phospholipids selected from the group consisting of dipalmitoylphosphatidylcholine (DPPC), phosphatidylcholine (PC; lecithin), phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS). Other suitable phospholipids further include distearoylphosphatidylcholine (DSPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidyglycerol (DPPG), distearoylphosphatidylglycerol (DSPG), dimyristoylphosphatidylglycerol (DMPG),

dipalmitoylphosphatidic acid (DPPA), dimyristoylphosphatidic acid (DMPA), distearoylphosphatidic acid (DSPA), dipalmitoylphosphatidylserine (DPPS), dimyristoylphosphatidylserine (DMPS), distearoylphosphatidylserine (DSPS), dipalmitoylphosphatidylethanolamine (DPPE), dimyristoylphosphatidylethanolamine (DMPE) and distearoylphosphatidylethanolamine (DSPE).

In a third aspect, there is provided a composition as defined above, wherein the liposomes comprise dipalmitoylphosphatidylcholine (DPPC).

In a fourth aspect, there is provided a composition as defined above, wherein the liposomes further comprise a sterol.

In a fifth aspect, there is provided a composition as defined above, wherein the MUC-1 peptide comprise an amino acid sequence of SEQ ID NO:1.

In a sixth aspect, there is provided a composition as defined above, wherein the MUC-1 peptide is lipidated at a lysine.

In a seventh aspect, there is provided a composition as defined above, wherein the MUC-1 peptide is palmitoylated.

In an eighth aspect, there is provided a composition as defined above, wherein the MUC-1 peptide comprises an amino acid sequence of SEQ ID NO:2.

In a ninth aspect, there is provided a composition as defined above, wherein the MUC-1 peptide is lipidated at the two final serines of SEQ ID NO:2.

In a tenth aspect, there is provided a composition as defined above, wherein the MUC-1 peptide is glycosylated.

In an eleventh aspect, there is provided a composition as defined above wherein the composition is lyophilized.

In a twelfth aspect, there is provided a composition that is reconstituted from the lyophilized composition as defined above.

In a thirteenth aspect, there is provided a composition as defined above, wherein the ratio of the flow rate of the stream of water to the flow rate of the stream of organic solvent is at least about 2:1.

In a fourteenth aspect, there is provided a composition as defined above, wherein the rate of cooling is on average at least about 4°C per hour.

In a fifteenth aspect, there is provided a composition as defined above, wherein the organic solvent stream is, prior to the mixing, at a temperature at least 10°C above the transition temperature of said lipids.

In a sixteenth aspect, there is provided a composition as defined above, further comprising providing means for inducing turbulence in the stream of water, the stream of organic solvent, or in a stream of mixture resulting from the mixing.

In a seventeenth aspect, there is provided a vaccine formulation comprising a sterile filtered composition as defined above.

In an eighteenth aspect, there is provided a vaccine formulation comprising a lyophilized composition as defined above.

In a nineteenth aspect, there is provided a vaccine formulation comprising a reconstituted composition as defined above.

In a twentieth aspect, there is provided a method for producing a preparation of liposomes of constrained particle size, said method comprising the steps of:

providing a substantially continuously flowing stream of water,

providing a substantially continuously flowing stream of an organic solvent, said organic solvent containing, dissolved therein, at least one lipid, said lipid or lipids being capable of forming liposomes, substantially continuously mixing said stream of water and said stream of organic solvent, so as to obtain a mixture, and

cooling said mixture, and

allowing liposomes to form within said mixture,

the ratio of the flow rate of the stream of water to the flow rate of the stream of organic solvent, and the rate of cooling of said mixture, being controlled so as to obtain a preparation of liposomes such that at least about 90% of the liposomes are of a particle size less than about 200 nm.

In a twenty first aspect, there is provided a composition as defined above; or a method as defined above substantially as hereinbefore described with references to the Examples.

It is the goal of the ~~invention~~disclosure to provide liposomes that are less than about 200 nm in size.

It was surprisingly found that, employing a method of liposome formation that includes mixing an organic liquid (wherein lipids are dissolved) and water, the concentration of organic solvent as well as rapid cooling of the resulting mixture are crucial for the formation and maintenance of consistent liposome size. The present method and apparatus facilitate the commercial and scalable synthesis of homogenous formulations of liposomally-incorporated drug vaccines by mixing a lipid solution, containing lipids dissolved in a water-miscible organic solvent, into flowing water under novel conditions to promote the continuous production of vaccine-quality liposomes. The method employs a continuous mixing system whereby the ratio of flow rates, i.e. ratio of lipid solution flow rate to water flow rate, is kept constant, thereby maintaining a constant percentage of organic solvent in the system. The method further employs a rapid and scale-independent cooling step, that follows

formation of liposomes and that prevents an increase in average liposome size. The method further provides an arrangement of pipes that promotes the formation of liposomes of desired size.

In order to produce liposomes that are less than about 200 nm in size, according to the present method the concentration of organic solvent in the organic solvent/water mixture is kept between 5% and 30%, more preferred, between 10% and 25%, most preferred between 10% and 25 %; the ratio of flow rates (water/organic solvent) is kept between 19:1 and 3 1/3:1, more preferably between 9:1 and 5:1 or between 9:1 and 4-1; and cooling of the liposome mixture is completed

(about 55°C to about 30°C) in less than 5 hours, more preferred less than 2 hours, most preferred less than 30 minutes, most preferably essentially instantly.

The ~~invention~~disclosure circumvents obstacles in the field, namely batch-to-batch inconsistency, undesired increase in liposome size during cooling, and the requirement for elaborate methods such as ultrasonication or pressurized systems. Liposomes produced according to the ~~invention~~disclosure are suitable for the production of vaccines for human or veterinary use.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic of the apparatus arrangement with insets depicting the arrangement of the "T"-junction and, optionally, whether a pipe comprises any internal protrusions or baffles to enhance turbulence and thereby facilitate mixing.

Figure 2 is a flow-chart depicting various parameters of the overall clinical manufacturing process.

Figure 3 is a photograph showing the convergence of dye (to mimic lipid/solvent) and water using different diameters of pipes: (A) 9 mm diameters for both pipes; (B) 5 mm (water) and 3 mm (lipid/solvent) pipes.

Figure 4 is a transmission electron microscopy photograph (18K magnification), showing the formation of liposomes carrying MUC-1 peptides using 20% t-butanol produced according to the present method.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present method is adaptable to large-scale, commercial production of formulations of nanoscale liposomes particularly of those that comprise substantially homogenous liposome particle sizes that are no bigger than about 200 nm in diameter. Preferred, more than 90% (volume weighted as determined by dynamic light scattering) of liposomes are less than about 200 nm, most preferred, more than 99% less than about 200nm. Such sized particles can be readily filter sterilized according to industry-approved clinical manufacturing standards.

A preparation of such homogenously-sized liposomes can be made according to the present invention by controlling the concentration of organic solvent, keeping it essentially constant at, and following, the formation of liposomes. By controlling