

not intending to be bound by this theory, it is believed that this results from the precise control of the flow conditions that are achieved in the microchannel format. In bulk systems, as the solvent mixes into the aqueous phase, it does so in an uncontrolled fashion, resulting in different sections of solvent diluting to their critical concentration for lipid solubilization under different applied mechanical shear stresses. This heterogeneity results in different liposome sizes, hence a more polydisperse sample. However, by reducing the length scale of the fluidic system, and taking advantage of low Reynolds number, laminar flow in a microfluidic network facilitates more controlled fluidic mixing on the length scale of the liposome. Specifically, the solvent reaches its critical concentration under the same applied shear stress, resulting in a more monodisperse population of liposomes.

[0015] A useful characteristic of liposomes is their ability to encapsulate (or perhaps excapsulate) ionic molecules from a surrounding aqueous medium. Thus, the invention includes embodiments wherein a reagent is included in the composition of lipids or lipid-forming materials and/or in the aqueous composition and at least a portion of the reagent is encapsulated (or excapsulated) in the liposomes. Examples of reagents which may be encapsulated in liposomes as part of the above-described methods include small molecules (for example, drugs, fluorescent molecules, amino acids) and large molecules (for example, proteins, peptides, DNA and RNA).

[0016] The lipid or lipid-forming materials used in the central microchannel to carry out the invention include all known materials for liposome formation. Examples of useful materials include combinations of phospholipid molecules and cholesterol. Particularly preferred are combinations of dimyristoylphosphatidylcholine, cholesterol, and dicetylphosphate. These materials are provided in a solvent which will dissolve the lipid or lipid-forming materials. The solvent must also be water miscible in order to diffuse into the aqueous composition. Examples of useful solvents include alcohols, such as isopropanol, methanol or ethanol. The lipids or lipid-forming materials are preferably provided in the solvent in a concentration of approximately 10 mM.

[0017] The aqueous composition is preferably an aqueous buffer solution, particularly preferably a phosphate-buffered saline solution, phosphate buffer, TRIS buffer or HEPES buffer.

[0018] By changing the length scale of the fluidics in which lipids self assemble into liposomes and simultaneously manipulating both the length scale and the shear forces applied to the vesicles upon formation, the invention advantageously provides fine control of liposome size and homogeneity. Particularly, liposome-containing compositions with liposomes having a mean diameter from 100 nm to 300 nm and a size distribution of 15 to 20% can be produced using the described methods. Microfluidics allows adjustment of the flow fields precisely using the simple principle of hydrodynamic focusing. This is a fundamental change in the way in which liposome vesicles are formed, thus enabling the production of monodisperse populations without the need for subsequent processing steps to modify liposome size.

[0019] The liposome self-assembly method described here can be used to provide liposomes for applications in on-

demand drug encapsulation and delivery and is readily scaled up using microfluidics with the development of multiplexed multichannel systems. We also predict that the synthesis and self-assembly of nanoscale particles for other applications in nanotechnology will also greatly benefit from adaptations of this approach.

[0020] The entire disclosure of all applications, patents and publications, cited herein and of U.S. Provisional Application No. 60/525,335, to which this application claims priority, is incorporated by reference herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1(a) provides a schematic of the self-assembly liposome formation process in a microfluidic channel. The color contours are generated by modeling of the flow field using Navier-Stokes convection and Stoke-Einstein diffusion analysis and represent the concentration ratio of isopropanol to the aqueous buffer. The lipid vesicles are formed where the concentration ratio of alcohol to buffer meets a critical condition when the lipids are no longer soluble in water, determined to be at 40 wt %. FIG. 1(b) provides a fluorescence micrograph of a microchannel network with input streams on the left side and output streams on the right side. FIG. 1(c) details the hydrodynamic focusing of the isopropanol stream at the first cross and FIG. 1(d) details the liposome collection and excess buffer extraction streams at the second cross.

[0022] FIG. 2(a) shows a three-dimensional color contour map of the fluorescence intensity of DiIC₁₈ at the hydrodynamically focused injection region of liposome formation. The intensity exhibits a ridge of increased fluorescence at the alcohol-aqueous buffer interface owing to increased efficiency of DiIC₁₈ fluorescence efficiency due to intercalation of the dye into the lipid bilayer. FIG. 2(b) shows the liposome mean diameter and standard deviation measured by light scattering vs. fluid flow velocity in center channel (The mean and standard deviation of the diameter of each of the liposome formulations is characterized by light scattering measurements (N4MD, Coulter Electronics Ltd.). For these measurements, a 0.1 wt % of Triton X-100 surfactant (Sigma) was added to the liposome formulations to reduce possible aggregation.) The isopropanol flow velocity is maintained at 2.4 mm/s while the flow velocity of each buffer channel is varied from 2.4 mm/s to 59.8 mm/s. The fluid flow velocity in the center channel on the x-axis is the sum of the flow velocities in each of the three input streams.

[0023] FIG. 3 provides optical micrographs of the fluid flow fields for the liposome self-assembly process. Flow profiles are imaged in the microchannel network with a fluorescence microscope (Axioplan 2, Carl Zeiss) using a halogen lamp as an excitation source with appropriate filters for excitation and detection for DiIC₁₈ (excitation 540 nm \pm 12.5 nm; beam splitter 565 nm; emission 605 nm \pm 27.5 nm) and carboxyfluorescein (excitation 470 nm \pm 20 nm; beam splitter 510 nm; emission 515 nm) and digitized using a CCD camera coupled to the top port of the microscope. FIG. 3(a) is a white light image showing the hydrodynamic focusing of the isopropanol injected into the aqueous buffer and its diffusion downstream; 3(b) is a fluorescence micrograph of DiIC₁₈ exhibiting its confinement within the liposome stream which has a much lower diffusion constant than isopropanol; and 3(c) is a fluorescent micrograph of water soluble carboxyfluorescein dye in the aqueous stream.