

and size of the liposomes formed was shown with the increase in the flow rate ratio from 500:1 to 1000:1 than from 1000:1 to 5000:1.

[0059] Aspects of the parallel flow device of the present disclosure are configured for a flow rate ratio of a flow of an aqueous stream from a first outlet of fluid introduction zone **14** to a flow of an organic stream from a second outlet of fluid introduction zone **14** between about 500:1 to about 10000:1, advantageously between about 1000:1 to about 7500:1, and more advantageously the flow rate ratio is about 5000:1.

[0060] A process for the formation of vesicles is disclosed herein. The process may comprise the steps of flowing an aqueous stream and flowing an organic stream centrally into and parallel with the flow of the aqueous stream, whereby the flowing aqueous stream completely sheaths or surrounds the flowing organic stream. A miscible organic material, flowing in the organic stream, may be dispersed with the aqueous stream and vesicles may form. The flowing of the aqueous stream and organic stream may comprise flowing the aqueous stream and the organic stream at a flow rate ratio between about 500:1 to about 10000:1. A device or process system may be adjustable and an adjustment to the flow rate ratio may be made by moving an outlet of the organic stream about a longitudinal axis of a sidewall, through which the aqueous stream is flowing, with respect to an inwardly tapered portion of the sidewall.

[0061] Aspects of the presently disclosed parallel flow device and methods may provide for the formation of liposomes that encapsulate reagents in a continuous 2-phase flow microfluidic network with precision control of size, for example, from 100 nm to 300 nm, by manipulation of liquid flow rates are described. By creating a solvent-aqueous interfacial region in a microfluidic format that is homogenous and controllable on the length scale of a liposome, fine control of liposome size and/or polydispersity may be achieved.

[0062] To best mimic biological systems, it may be desirable to create environments that are controllable on the dimension of the particle itself to elicit fine control of nanometer scale synthesis and self-assembly processes. Therefore, aspects of the present disclosure may provide for the formation of liposomes in microfluidic systems, the characteristics of fluidic flow in a micrometer-scale parallel flow device be used to precisely control the distribution of chemical conditions and mechanical forces so that they are substantially constant on a length scale equivalent to that of a liposome. Hence, forming liposomes in a micrometer-scale flow field may result in more homogenous conditions during liposome self-assembly and resultant liposome populations that are more uniform in size, hence of low polydispersity.

[0063] Thus, the present disclosure includes methods for producing a liposome-containing composition, which includes: providing a solvent stream of a composition of lipids or lipid-forming materials dissolved in a solvent through a central microchannel having a hydrodynamic diameter of 100 μm or less, preferably 70 μm or less; and centrally entering a solvent stream into a parallel flowing aqueous stream of an aqueous composition which hydrodynamically focuses the solvent stream and forms an aqueous sheath about the solvent stream having an interfacial region where the solvent stream and the aqueous stream disperse or diffuse into each other to provide conditions such that liposomes self-assemble from the lipids or lipid-forming materials.

[0064] When the two liquid phases come into contact, the solvent phase and aqueous phase may rapidly diffuse into one another. The flow rates of the solvent and aqueous streams may be adjusted to control the degree of hydrodynamic focusing and ultimately the liposome size. The lipids self-assemble where the concentration of the solvent phase containing the lipid or lipid-forming materials and the aqueous composition is at a critical condition where lipids are no longer soluble and thus self-assemble into liposomes. The formed liposomes may remain centrally in a microchannel or tube because: (i) liposomes formed along the interfacial region may follow stream lines and may be directed to collect at the center point in the channel; and (ii) at this point the solvent may have diluted to a concentration where it can no longer solubilize any fraction of the lipid.

[0065] One may control the liposome size by altering the ratio of the flow rate in the sheathing inlet channel(s) compared to the central inlet channel. This may result in a decrease or increase in both the mean and range (polydispersity) of liposome diameter. Thus, by tuning of the flow rates in the inner and outer parallel flow streams, the physical characteristics of the resultant liposome preparation may be changed or controlled.

[0066] A useful characteristic of liposomes is their ability to encapsulate (or perhaps excapsulate) ionic molecules from a surrounding aqueous medium. Thus, the present disclosure 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, polymers, DNA and RNA).

[0067] The lipid or lipid-forming materials used in the central feed line **20** to make liposomes 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 dicytlylphosphate. These materials may be provided in a solvent that will dissolve the lipid or lipid-forming materials. The solvent may also be water miscible in order to diffuse or disperse into the aqueous composition. Examples of useful solvents include alcohols, such as isopropanol, methanol or ethanol. The lipids or lipid-forming materials may be provided in the solvent in a concentration of approximately 10 mM-50 mM.

[0068] The aqueous composition may be an aqueous buffer solution, particularly a phosphate-buffered saline solution, phosphate buffer, TRIS buffer or HEPES buffer. By changing the length scale of the vesicle formation zone **12**, flow rate ratio of aqueous solution to organic solution, and/or diameter of central line **20**, fine control of liposome size and homogeneity may be provided. Particularly, liposome-containing compositions with liposomes having a mean diameter from about 10 nm to about 300 nm and a size distribution of 15 to 20% may be produced using the herein described devices and methods. The parallel flow device of the present disclosure may provide for the adjustment of the flow fields using the simple principle of hydrodynamic focusing, thus enabling the production of substantially monodisperse populations without the need for subsequent processing steps to modify liposome size.