

point in the channel; and (ii) at this point the alcohol has diluted to a concentration where it can no longer solubilize any fraction of the lipid. According to two-dimensional modeling of this flow field with Navier-Stokes convection and Stoke-Einstein diffusion analysis, this increase in fluorescence intensity corresponds to an isopropanol concentration of approximately 40 wt %.

[0033] One can control the liposome size by altering the ratio of the flow rate in the side inlet channels compared to the center inlet channel. As the bulk liquid flow rate in the center channel downstream of the first cross increases, the magnitude of the shear stresses applied to the liposomes as they self assemble also increases. This results in a decrease in both the mean and range (polydispersity) of liposome diameter, as can be seen in **FIG. 2b**. Thus, by tuning of the flow rates in the microfluidic channel, the physical characteristics of the resultant liposome preparation can be readily controlled, for example, over the range of 100 nm to 300 nm. Further, the liposome preparations are more monodisperse in size than liposomes prepared by traditional bulk methods. Although 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 isopropanol mixes into the aqueous media, it does so in an uncontrolled fashion, resulting in different sections of alcohol 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 (M. C. Woodle, D. Papahadjopoulos, *Meth. Enzymol.* 171, 193 (1989)). 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 diffusion-based mixing on the length scale of the liposome. Specifically, the alcohol reaches its critical concentration under the same applied shear stress, resulting in a more monodisperse population of liposomes.

[0034] A useful characteristic of liposomes is their ability to encapsulate (or perhaps excapsulate) ionic molecules from a surrounding aqueous medium. Thus, functional characterization of liposomes self-assembled in a microfluidic channel is of interest. **FIG. 3** shows three different images of the same microfluidic flow field. In panel (a), the microchannel network is viewed with transmitted light; immediately apparent is the visible refractive index change at the interface between the alcohol and aqueous phases that dissipates as the two phases interdiffuse. Panel (b) shows the microfluidic network under fluorescent imaging conditions appropriate to visualize DiIC₁₈ that is present as 1 wt % of the lipid fraction in the alcohol phase. The hydrodynamic focusing of this stream is clearly visible in this micrograph. It is also interesting that the DiIC₁₈ stream does not get progressively wider as the alcohol-aqueous boundary does as it migrates down the microchannel. This is due to the incorporation of the low molecular weight DiIC₁₈ into liposomes, which have a very low diffusion coefficient; and therefore diffuse minimally in the radial direction on the time frame of the liquid flowing through the microchannel. Panel (c) shows the microfluidic network under fluorescent imaging conditions appropriate to visualize carboxyfluorescein dye, which is present in the aqueous stream at a concentration of 1 mM. Here the aqueous streams can be seen focusing the alcohol stream. Careful inspection of this panel shows that the aqueous carboxyfluorescein dye does

diffuse into the center region, showing that the carboxyfluorescein that is not encapsulated in liposomes still diffuses freely within the microfluidic channel.

[0035] Liposomes that self assemble in the microchannel presented in **FIG. 3** should then have an aqueous interior that contains carboxyfluorescein surrounded by a lipid membrane with DiIC₁₈. Thus, two-color fluorescent imaging of a liposome should reveal the interior and membrane portions of the assembly. **FIG. 4** shows a single liposome collected from this microfluidic flow network and nonspecifically adhered to a glass coverslip. Panel (a) shows the liposome with a fluorescence microscopy appropriate to visualize the DiIC₁₈ incorporated into the membrane, while panel (b) shows the same liposome visualized under the same magnification with fluorescence conditions to visualize carboxyfluorescein. The two images coincide in their spatial position and are stable over the time frame of several minutes as would be expected if the object in view is a contiguous membrane that encapsulates a continuous aqueous interior.

[0036] The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

[0037] From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention and, without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

We claim:

1. A method for producing a liposome-containing composition, which comprises:

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, and

impinging on said solvent stream through at least one side microchannel at least one aqueous stream of an aqueous composition which hydrodynamically focuses the solvent stream and forms a stream within the central microchannel having an interfacial region where the solvent stream and the at least one aqueous stream diffuse into each other providing conditions where liposomes self-assemble from the lipids or lipid-forming materials.

2. The method of claim 1, 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 said reagent is encapsulated in the liposomes.

3. The method of claim 1, wherein the liposome-containing composition contains liposomes having a mean diameter of from 100 nm to 300 nm and a size distribution of less than 20%.

4. The method of claim 1, wherein at least two aqueous streams are provided by at least two side microchannels, which at least partially oppose one another, and which impinge on the solvent stream to hydrodynamically focus it.

5. The method of claim 1, wherein two aqueous streams are provided by two side microchannels each at a 90° angle or less to the solvent stream, and opposite one another, which impinge on the solvent stream to hydrodynamically focus it.