

[0024] FIG. 4 provides two-color confocal fluorescence imaging of a single liposome formed in flow conditions of 0.5 $\mu\text{L}/\text{min}$ flow rate of the isopropanol stream and 20 $\mu\text{L}/\text{min}$ flow rate of the aqueous buffer streams. Two-color fluorescence imaging of liposomes showing analyte encapsulation were obtained using a confocal fluorescence imaging system (LSM 5 Pascal, Carl Zeiss) with a 40 \times objective and 10 \times digital zoom. The DiIC₁₈ liposome image was measured with a laser excitation of 543 nm, through a 543 nm beam splitter, and emission was detected through a 560 nm low pass filter. The carboxyfluorescein liposome image was measured with a laser excitation of 488 nm passing through a 488 nm beam splitter, and emission was detected through a 530 nm low pass filter. The flow conditions in this example produce a liposome mean diameter of 133 nm and standard deviation of 26 nm determined by light scattering measurements. FIG. 4(a) is a fluorescence image for DiIC₁₈ that exhibits intercalation of the dye into the lipid bilayer; and 4(b) is a fluorescence image for carboxyfluorescein (CF) that is encapsulated in the liposome's aqueous interior.

[0025] Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

[0026] In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and, all parts and percentages are by weight, unless otherwise indicated.

EXAMPLES

[0027] A stream of lipid tincture is hydrodynamically focused at a microchannel cross junction between two aqueous buffer streams each impinging on the lipid stream at 90°.

[0028] The lipid tincture contains dimyristoylphosphatidylcholine (DMPC) and cholesterol in a molar ratio of 1:1 diluted in chloroform solvent (all from Aldrich) with 1 wt % of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiIC₁₈) added for fluorescent imaging. The chloroform solvent was evaporated under a stream of nitrogen at room temperature to form a lipid film on the bottom of a test tube. The test tube was then placed in a vacuum desiccator for at least 24 h to ensure dryness. The dried lipid mixture was resolubilized with 500 μL dry isopropanol yielding a 10 mM concentration of lipid solution.

[0029] To provide the microchannel cross junction, a microchannel network (200 μm channel width) was anisotropically etched on the surface of a silicon wafer (<100> orientation, 75 mm diameter, 0.3 mm thickness, from Virginia Semiconductor) to a depth of 40 μm through a photolithographically patterned thermal SiO₂ (100 nm) hard mask using tetraethylammonium hydroxide (TEAH, Alfa Aesar) (1:2 dilution 25% w/w aqueous TEAH solution, 80° C.). The same procedure was followed to etch fluid through-holes on the back-side of the wafer that were aligned to the microchannel network in the front side. Following this, all surface oxide was removed (6% buffered HF etch) and then re-oxidized to form a 100 nm thick SiO₂ film encapsulating the silicon substrate. Finally, a glass cover wafer (75 mm diameter 0.1 mm thickness, Corning 7740) was anodically

bonded to the front surface of the silicon wafer using a 580 V applied potential and heated to 400° C. to seal the microchannel network.

[0030] Following a procedure analogous to the bulk procedure that is described in L. Locascio-Brown, A. L. Plant, V. Horvath, *Anal. Chem.* 62, 2587 (1990), isopropyl alcohol containing the dissolved lipids is flowed through the center inlet channel, and an aqueous phosphate buffered saline solution flows through each of the two side inlet channels. Reagent transfers can be accomplished using gastight glass syringes interfaced to the microchannel network through capillary tubing (PEEK Tubing, Upchurch Scientific) and capillary connectors (Nanoports, Upchurch Scientific) that are bonded to the fluid through-holes etched in the silicon wafer. Programmable syringe pumps (Harvard Apparatus) can be used to control the fluid flow rates by computer, for example, using a LabVIEW software interface. Phosphate buffered saline solution (10 mM phosphate, 27 mM potassium chloride, 137 mM sodium chloride, pH=7.4) is used as the hydration buffer. For encapsulation experiments, a 1 mM carboxyfluorescein solution is prepared in this buffer. The liposome formulations (100 μL sample size) are collected at each flow condition in polycarbonate cuvetts. After collection, 1 mL of phosphate buffered saline solution (10 mM) is added to each formulation and sealed for further characterization.

[0031] When the two liquid phases come into contact, the isopropanol rapidly diffuses into the aqueous phase and vice versa. The flow rates of the alcohol and buffer channels can be adjusted to control the degree of hydrodynamic focusing and ultimately the liposome size. The lipids self-assemble where the concentration of alcohol and buffer mixture is at a critical condition where lipids are no longer soluble and thus self-assemble into liposomes. Finite element modeling of this 2-phase flow process schematically presents the general approach to microfluidic self-assembly of liposomes in a microfluidic network (see FIG. 1a). A fluorescence image of the device used in these experiments is shown in FIG. 1b, with a close-up view in FIG. 1c of the hydrodynamically focused isopropanol stream where liposome formation occurs. Immediately downstream of the first cross intersection, the fluorescent intensity of the center stream increases sharply indicating the formation of the lipids into liposomes (the quantum efficiency of the fluorescent dye in this experiment (DiIC₁₈) increases dramatically upon incorporation into a lipid membrane). The liposomes then flow to the second cross intersection as a tightly focused stream owing to the low Reynold's number laminar flow typical of microfluidics and the low diffusion coefficient of liposomes. At this second cross (FIG. 1d) excess buffer is removed via the two side outlet channels and the liposome suspension is collected via the center outlet channel.

[0032] The liposomes form (as is manifested by the increased fluorescence of the DiIC₁₈) along the boundary between the isopropanol and water, as can be seen in FIG. 2a. A ridge of increased fluorescence is clearly visible as the aqueous streams focus the isopropanol. Fluorescence intensity increases to its maximum value immediately downstream of the minimum width of the isopropanol stream indicating the highest concentration of liposomes. Two effects lead to the high liposome concentration here in the system: (i) liposomes formed along the interfacial region follow stream lines and are directed to collect at the center