

dispersion of alcohol is exhibited through the co-flowing streams. FIG. 5 shows that the outer flowing aqueous stream sheathes and focuses the central alcohol stream. In this respect, a mutual diffusion of miscible fluids, in an alcohol stream and a aqueous stream, may enable the assembly of spherical vesicles at the solvent interface due to a change in amphiphile solubility.

[0050] FIG. 6 shows the dispersion of an organic material at specific cross-sections of a vesicle formation zone 16. Flowing an organic material containing stream 23, through central feed line 20, and into aqueous stream 25, via outlet 22, may provide a substantially controlled and uniform dispersion of the organic material with the aqueous stream. Outer sheathing of organic stream 23, with aqueous stream 25, may create physicochemical conditions in the aggregate co-flowing streams that are substantially radially symmetric. The vesicle formation device may be configured to provide a controlled and substantially uniform dispersion of an organic material and ethanol/buffer, in organic stream 23, with aqueous stream 25 at a plane perpendicular to vesicle formation zone 16, as shown in cross-sectional diagrams 30-34.

[0051] Vesicle formation zone 16 may be configured and disposed to receive a parallel flow of the outer flow stream 25, flowing from a first outlet, sheathing the inner flow stream 23, flowing from a second outlet, and may be configured for a controlled and substantially uniform dispersion of an organic material, flowing in the inner flow stream, at a plane perpendicular to vesicle formation zone 16.

[0052] Cross-sectional concentration diagrams 30-34 data were rendered from data generated with a finite-element numerical modeling software package that use a creeping-flow (i.e. low Re flow) limit of the Navier-Stokes equations coupled with a convection-diffusion equation. A alcohol/water mixture may have a viscosity that is great than either liquid in pure form, therefore a plot of viscosity as a function of alcohol concentration in water may have a roughly parabolic shape with a maxima at ~60% EtOH in water. Hence, the diffusion coefficients may have a minima at the same concentration conditions. A 5th order polynomial fit of data was employed to account for the viscosity of alcohol/water mixture and any heats of mixing were neglected as they may be negligible.

[0053] Cross-sectional diagram 30 shows an initial ethanol concentration, with ethanol being delivered through central line 20, in fluid flowing through outer sheath 12, adjacent exiting outlet 22. Cross-sectional diagrams 31, 32, 33, and 34 show ethanol concentrations in fluid flowing through outer sheath 12 at 0.1 mm, 0.2 mm, 1.0 mm, and 4.0 mm, respectively. It is shown that the dispersion of the alcohol flowing from outlet 22 into the flow of fluid in outer sheath 12 is greatest adjacent to outlet 22. As the fluids proceed from outlet 22, the rate of dispersion decreases. The rate of decrease in dispersion along the length of outer sheath 12 may indicate that mass diffusion, as opposed to mixing of the miscible fluids, may be the predominate mechanism driving the dispersion of alcohol. Therefore, the parallel flow device for making vesicles of the present disclosure may provide for a controlled dispersion of an organic material with an aqueous solution and provide a control production of liposome size and/or polydispersity.

[0054] FIG. 7 graphically shows the size of liposomes formed with the device and method of the present disclosure, shown as annular device, as compared to other devices and methods. As shown in FIG. 7, the polydispersity of the size of

liposomes may be significantly less than the polydispersity of size of liposomes obtained by film hydration/membrane extrusion and planar microfluidic devices. This is represented with the larger height showing a greater differential number fraction and the narrowness of the curve showing a lower range of geometric radius.

[0055] FIG. 8 graphically shows liposome size with respect to selected diameters of a feed line inlet 22 feeding an organic material to the vesicle formation zone 16 of the parallel flow device for the formation of vesicles of the present disclosure. An organic/alcohol stream was fed into vesicle formation zone 16 with different feed lines, each having a different inner diameter. Specifically, a first feed line 20 and outlet 22 had an inner diameter of 65 μm ; a second feed line 20 and outlet 22 had an inner diameter of 125 μm ; and a third feed line 20 and outlet 22 had an inner diameter of 255 μm . As shown in FIG. 8, the size, or geometric radius, and the polydispersity, or differential number fraction, of the liposomes may be controlled with different sizes or inner diameters of feed lines 20 and outlets 22. In each example shown, each feed line 20 had an equivalent inner diameter as its outlet 22. The results show that a smaller inner diameter results in less polydispersity and smaller size liposomes.

[0056] In aspects of the present disclosure, second outlet 22 of fluid introduction zone has an inner diameter of at most 255 μm , advantageously at most 125 μm , and more advantageously at most 65 μm .

[0057] FIG. 9 graphically shows liposome size with respect to a center organic/lipid containing line 20 inserted at varying distances beyond the termination of a multi-line assembly 24 containing an aqueous phase, in the parallel flow device of FIG. 1. Liposome preparations were made with the center organic/lipid containing line 20 inserted 5 mm, 10 mm and 20 mm beyond the plane of termination of outer feed lines 24, or inlets 27, of the multi line assembly containing the aqueous phase. The operation conditions were: center line ID=65 μm ; volumetric flow rate ratio=5,000:1; aqueous flow rate=1000 $\mu\text{L}/\text{min}$; and organic/lipid flow rate=0.2 $\mu\text{L}/\text{min}$. The plots in FIG. 9 show that increasing the distance between outlets 22, of central line insert 20, and outlets 27, of outer feed lines 24, provides liposomes having less polydispersity. This may be due to eddy currents being present in the aqueous flow stream adjacent outlets 27. Having outlet 22 further downstream may provide a more stable axial flow of the aqueous fluid flowing from outlets 27 prior to the introduction of the organic stream through outlet 22.

[0058] FIG. 10 graphically shows liposome size with respect to varying flow rate ratios of an organic stream with an aqueous stream. Varying the flow rate ratios may be accomplished by varying the pumping rate of fluids into inlets 10 and/or 11 or by longitudinally moving central feed line 20 to dispose outlet 22 at a different point in inwardly tapered portion 15 of outer sheath 12, as shown in vesicle forming device 300 in FIG. 3. It is shown in FIG. 10 that varying the flow rate of the aqueous stream with respect to the flow rate of the organic stream may control liposome size and polydispersity. For example, increasing the flow rate ratio (aqueous flow rate:organic flow rate) from 500:1 to 1000:1 significantly decreases both polydispersity and size of the liposomes formed. A further increase of the flow rate ratio to 5000:1 further decreases both polydispersity and size of the liposomes formed. A greater decrease in both polydispersity