

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 to provide conditions such that liposomes self-assemble from the lipids or lipid-forming materials. In a preferred embodiment, at least two aqueous streams provided by at least two side microchannels, which at least partially oppose one another, impinge on the solvent stream to hydrodynamically focus it. More preferably, two aqueous streams provided by two side microchannels each at a 90° or less angle to the solvent stream, and opposite one another, impinge on the solvent stream to hydrodynamically focus it. However, other means of providing one or more side streams to focus the solvent stream and provide the interfacial region for liposome formation may be used.

[0008] For collection of the formed liposomes, it can be useful to provide, downstream of the region where the liposomes form, at least one side outlet microchannel for removing non-liposome materials from the central microchannel. The formed liposomes will flow in the central microchannel, typically as a tightly focused stream in the center area of the channel, owing to the low Reynold's number laminar flow typical of microfluidics and to the low diffusion coefficient of the formed liposomes. The outlet can be simply provided by one or more branching side microchannels. Material outside the diameter of the tightly focused stream of formed liposomes will be removed by such outlet channels facilitating isolation of a liposome suspension formed in the center of the central microchannel. In a preferred embodiment, non-liposome materials are removed from the central microchannel by two side outlet microchannels angled at 90° or more to the central microchannel and on opposite sides thereof. Other means for separating the formed liposomes can also be used.

[0009] Although any devices which provide the necessary microfluidics can be used to carry out the method, preferably, the device for carrying out the method is in the form of a silicon wafer containing the microchannels in the desired pattern to provide the central microchannel, side microchannels and outlet microchannels, as desired. Known photolithography techniques can be used to pattern microchannels in silicon materials. For example, the microchannels may be fabricated in the silicon chip with a transparent wafer, e.g., glass, cover using a two-step photolithography process in combination with an anisotropic wet-chemical etch (TMAH), followed by anodic bonding. In a first photolithographic step, through-holes for fluidic access to the device are patterned in the back of the silicon chip. In a second step, the microchannel pattern is formed on the front of the chip. After the patterning is complete, the silicon chips are cleaned, and a wet oxide layer (e.g., a 500 nm thick SiO₂ film) is grown on the surface. Finally, the channels are sealed by a covering using an anodic bonding technique: for example, a 0.5 mm thick borosilicate (Pyrex) wafer is brought into contact with the front of the silicon chip, and the silicon/Pyrex sandwich is heated to, e.g., 400° C., with an applied voltage. Modifications may be made to these photolithography, etching and anodic bonding methods according to the knowledge in these arts.

[0010] In one embodiment, the microchannels are formed in a material transparent on at least one side to allow

observation of the microchannel, e.g., with the glass cover construction discussed above. This allows observation of the flow and liposome formation in the microchannels. For this purpose, it can be advantageous to provide a fluorescing material in the solvent stream and/or the aqueous streams to allow fluorescent observation and/or imaging of the liposome formation through the transparent cover.

[0011] The microchannels are preferably etched on the surface of a silicon wafer to a width and depth of 10 to 200 μm, more preferably 20 to 160 μm. The entire length of the central microchannel is preferably about 10 to 30 mm. The fluid through-holes are etched preferably of the same width as the microchannels or larger on the back-side of the wafer and aligned to the microchannel network for input and output ports. The dimensions of the entire silicon chip with cover device are preferably about 20 mm to 50 mm long and 15 mm to 30 mm wide with a thickness of 0.7 mm to 1 mm.

[0012] The flow of the solvent and aqueous streams through the device is preferably accomplished by pumping of the streams into the central and side microchannels, respectively. However, other fluid driving methods, such as electrically driven pumping could be used. In a preferred embodiment, pumping is accomplished by using gastight glass syringes interfaced to the microchannel network through capillary tubing and capillary connectors that are bonded to the fluid through-holes etched in the silicon wafer. Programmable syringe pumps can be used to control the fluid flow rates by computer, for example, using a LabVIEW software interface. Fine control of the respective flow rates is advantageous for providing fine control over the size of the resulting liposomes. Preferably, the central microchannel is provided with a flow rate of the solvent phase of about 1 to 12 mm/s and the side microchannels, when two are provided, of about 10 to 100 mm/s.

[0013] When the two liquid phases come into contact, the solvent phase and aqueous phase rapidly diffuse into one another. The flow rates of the solvent and aqueous streams can 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 remain in the center of the microchannel because: (i) liposomes formed along the interfacial region follow stream lines and are directed to collect at the center point in the channel; and (ii) at this point the solvent has diluted to a concentration where it can no longer solubilize any fraction of the lipid.

[0014] 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. Thus, by tuning of the flow rates in the microfluidic channels, the physical characteristics of the resultant liposome preparation can be readily controlled, for example, over the range of 100 nm to 300 nm diameter. Further, the liposome preparations are more monodisperse in size than liposomes prepared by traditional bulk methods. Although