

**CONTROLLED VESICLE SELF-ASSEMBLY IN  
CONTINUOUS TWO PHASE FLOW  
MICROFLUIDIC CHANNELS**

**[0001]** This application claims priority to U.S. Provisional Application No. 60/525,355, filed Nov. 26, 2003.

**[0002]** Methods 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 polydispersity can be achieved. Traditional liposome preparation methods are based on mixing of bulk phases, leading to inhomogeneous chemical and/or mechanical conditions during formation; hence liposomes prepared by those methods are often polydisperse in size and lamellarity.

**[0003]** There are a growing number of applications for nanoscale particles in biology that include interrogating (see, for example: E. J. Park, M. Brasuel, C. Behrend, *Anal. Chem.* 75, 3784 (2003); A. S. Arbab, L. A. Bashaw, B. R. Miller B R, *Transplantation* 76, 1123 (2003); M. E. Akerman, W. C. W. Chan, P. Laakkonen, *Proc. Natl. Acad. Sci. U.S.A.* 99, 12617 (2002); M. Bruchez, M. Moronne, P. Gin P, *Science* 281, 2013 (1998); W. C. W. Chan, S. M. Nie, *Science* 281, 2016 (1998); B. Dubertret, P. Skourides, D. J. Norris, *Science* 298, 1759 (2002)), perturbing (see, for example: H. E. Sparrer, A. Santoso, F. C. Szoka, *Science* 289, 595 (2000); and I. Koltover, T. Salditt, J. O. Radler, *Science* 281, 78 (1998)) and stimulating (see, for example: A. K. Salem, P. C. Searson, K. W. Leong, *Nat. Mater.* 2, 668 (2003)) the cellular environment. The design and production of nanometer scale objects, such as quantum dots, colloidal particles, and vesicles, can be accomplished in bulk either by chemical synthesis or self-assembly processes. In the cellular factory, chemical synthesis and self-assembly processes are exquisitely controlled by the closely-regulated local environment to ensure the reproducible production of nanometer-scale components such as proteins and vesicles. In bulk production methods, the local environment is not well controlled leading to significant chemical fluctuations, or electrical, mechanical perturbations that often result in inhomogeneous populations of nanoparticles.

**[0004]** Liposomes (see, e.g., A. D. Bangham, M. M. Standish, J. C. Watkins, *J. Mol. Biol.* 13, 238 (1965)) are one example of nanoparticles that have been used for a wide variety of biological applications including targeted drug delivery and DNA transfection (see, e.g.: G. Gregoriadis, *Liposome Technology Volume 3; Targeted Drug Delivery and Biological Interactions* (CRC Press, Boca Raton, 1983); and D. D. Lasic, D. Papahadjopoulos, *Science* 267, 1275 (1995)). Liposomes are cellular mimetics composed of a lipid bilayer membrane that encapsulates and sequesters species inside from species residing outside the membrane. Of critical importance to the successful implementation of liposomes in vivo is the ability to control the liposome size and size distribution, as size influences the clearance rate from the body and ultimately determines the drug dosage. Conventional modes of liposome preparation require the mixing of two or more phases, typically liquid-liquid or liquid-solid, resulting in the spontaneous self-assembly of

the lipid mixture into a spherical bilayer membrane (see, e.g.: G. Gregoriadis, H. da Silva, A. T. Florence, *Int. J. Pharm.* 65, 235 (1990); F. C. Szoka, D. Papahadjopoulos, *Proc. Natl. Acad. Sci. U.S.A.* 75, 4194 (1978); C. Pidgeon, S. McNeely, T. Schmidt, *Biochem.* 26, 17 (1987); H. Hauser, *Biochim. Biophys. Res. Commun.* 45, 1049 (1971); S. Batzri, E. D. Korn, *Biochem. Biophys. Acta.* 298, 1015 (1973); T. H. Fischer, D. D. Lasic, *Mol. Cryst. Liq. Cryst. Lett.* 102, 144 (1984); H. Kikuchi, H. Yamauchi, S. Hirota, *Chem. Pharm. Bull.* 39, 1522 (1991); A. Wagner, K. Uhl-Vorauer, G. Kreismayer, *J. Lip. Res.* 12, 259 (2002); T. S. Aurora, W. Li, H. Z. Cummins, *Biochimica et Biophysica Acta* 820, 250 (1985); P. L. Luisi, P. Walde, *Giant Vesicles* (John Wiley & Sons, Chichester, 2000)). These self-assembly processes typically occur in a system with a characteristic length on the order of centimeters, resulting in chemical and/or mechanical conditions that are highly heterogeneous on the length scale of a liposome. Thus, a given liposome may experience any one of many different sets of mechanical and chemical conditions during its self-assembly, often leading to liposome preparations with large polydispersity with respect to size and lamellarity.

**[0005]** To best mimic biological systems, it is 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. As an aspect of the invention, it was discovered that several characteristics of microfluidic systems provide the ability to accomplish process control at this level. First, in these microfluidic systems, interfacial forces dominate and bulk inertial forces are typically negligible, leading to enhanced heat and diffusional mass transfer properties. Second, the laminar flow conditions in microfluidic channels can be used to create a well-defined and predictable interfacial region between two fluids. This characteristic has in fact been used to focus fluid streams hydrodynamically to submicrometer dimensional scales for rapid mixing (see, e.g., J. B. Knight, A. Vishwanath, J. P. Brody, *Phys. Rev. Lett.* 80, 3863 (1998)) and patterning (e.g.: P. J. A. Kenis. R. F. Ismagilov, G. M. Whitesides, *Science* 285, 83 (1999)). These properties of microfluidics allow control of chemical processes on nanometer length scales that were previously difficult to access experimentally.

**[0006]** According to the invention for the formation of liposomes in microfluidic systems, the characteristics of fluidic flow in a micrometer-scale channel can be used to precisely control the distribution of chemical conditions and mechanical forces so that they are constant on a length scale equivalent to that of a liposome. Hence, forming liposomes in a micrometer-scale flow field results in more homogenous conditions during liposome self-assembly and resultant liposome populations that are more uniform in size, hence of low polydispersity. Theoretical analysis of the laminar flow field engendered by microchannel flow when three microfluidic channels intersect show that the distribution of chemical species within the microfluidic network is constant and predictable (see, e.g., **FIG. 1a**, further discussed below).

**[0007]** Thus, the invention 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  $\mu\text{m}$  or less, preferably 70  $\mu\text{m}$  or less; and impinging on