Microfluidic synthesis of liposome for drug delivery system

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INTRODUCTION

Liposomes were first discovered by Bangham in 1965, meaning lipid body and defined as a spherule/vesicle of lipid bilayer enclosing an aqueous compartment. Basic components of liposome are phospholipids and cholesterol. Phospholipids are amphiphilic surfactants which forms bilayer/vesicle upon hydration. Thus liposomes are spherical self-closed structures, composed of curved lipid bilayer, which enclose part of the surrounding solvent into their interior. The size of a liposome ranges from some 20 nm up to several micrometers and they may be composed of one or several concentric membranes, each with a thickness of about 4 nm (Lasic 1993). Liposome offer great potential in drug delivery system, like versatility in terms of size and electrical charge, ability to encapsulate both hydrophilic (in aqueous core) and lipophilic drugs (in lipid bilayer), relative non toxicity as compared to other carrier systems, ability to escape macrophages in the lungs (stealth liposome), antibodies can be covalently coupled to liposome to ensure their cell specificity.

In all conventional methods for example thin film hydration or Bangham method, reverse phase evaporation (Szoka et al 1978), detergent depletion, freeze-thawing etc; common three or four steps are involved which are (1) Drying of lipid film from organic solvent, (2) Dispersion of lipid in aqueous media, (3) Sizing, (4) Purification of resultant liposome and analysis of final product. All these suffers from the following problems- poor scalability on industrial scale, use of high shear forces and organic solvents may lead to contamination of product, large time require for batch, very less encapsulation of solute, high cost. This illustrates the need for novel liposome preparation techniques that can overcome these problems. One can think of a microfluidic synthesis of liposome, where liposomes are formed at interfaces formed in microchannels using “hydrodynamic focusing” principle (Jahn et al 2004, 2007). In this method the stream of lipid dissolved in alcohol is focused between aqueous streams (generally drug dissolved in phosphate buffer saline). At the two liquid interfaces, amphiphilic lipid molecules arranges and self assembles in to vesicle encapsulating aqueous phase.

MATERIAL AND METHODS

The polymer polydimethyl siloxane (PDMS) alongw with curing agent was purchased from Dow corning (Sylgard 184). Slender nylon thread of diameter 220 µm was used as template for generating channels in PDMS. Chloroform purchased from SD- fine; used for swelling of PDMS. Microscope glass slide (1x3 inches W x L) was used as rigid substrate. Stainless steel tubing (16 gauge), needle (18 gauge) and 10 ml gastight syringe (Hamilton, Swiss) were used as connectors. Hydrogenated soya phosphatidylcholine (SPC-3) received as a gift sample from Lipoid Inc, used for synthesis of liposome. Ethanol and all salts require for PBS (all AR grade) were purchased from SD-Fine chemicals, India.

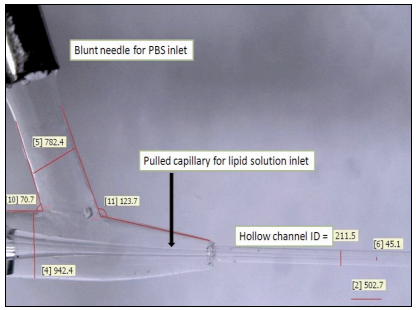
Fabrication of microfluidic device: Jeong W. et al fabricated very simple and inexpensive microfluidic device (MF) for generation of microparticles. Same device with some modification was fabricated. Nylon thread was used to create hollow, monolithic microchannel in PDMS (Verma

et al. 2006). After curing PDMS, the slab was swelled by deeping it in chloroform solution for 2 hours, the thread was removed out and pulled capillary was inserted to form axial geometry MF device. Figure 1a shows the photograph of axial geometry MF device. The diameter of glass capillary at pulled end is 45µm (ID30µm) and that of hollow microchannel is 210 µm. The total length of channel after mixing of two streams is 4 cm. Device performance was checked by laminar flow experiment using fountain pen ink and fluorescent dyes Rhodamine-B using fluorescence microscope (Nikon TE 2000-U, equipped with Nikon DS Fi1camera, Japan and NIS element software)

Liposome Synthesis and characterization : Liposomes were synthesized by passing phospholipid (soya phosphatidylcholine 5mg/ml) dissolved ethanol stream through pulled capillary (central) and phosphate buffer saline through side channel (figure 1a). Gastight syringe was mounted on syringe pump (NE 1000, New Era Syringe Pump Systems Inc.) and SS tubing was used as macro to micro interface connectors. The resultant liposomes were characterized by dynamic light scattering/DLS/PCS (Zetasizer nano ZS, Malvern, UK) and transmission electron microscopy (Philips CM 200, operating voltage 20-200 kv, resolution 2.4 A^{o}).

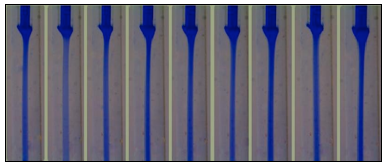
RESULTS AND DISCUSSION

Axial focusing and theoretical estimate of focused stream width : Figure 1b shows the effect of constant flow rate ratios (FRR) of two fluids on width of focused stream. The ink flow rate from left to right was changing 1µL/min to 9 µL/min and accordingly the volumetric flow rate of water was changed (50 – 450 µL/min), keeping ratio (ink: water) constant at 50. This image clearly shows that the width of central ink stream is constant (43 +/- 1.5µm). Figure 1c is fluorescent image of changing ratio of flow rates. The central Rhodamine-B flow rate was kept constant at 5 µL/min. From L to R the FRR is changing from 20 to 150 with increment of 10.



1a)

1b)



1c)

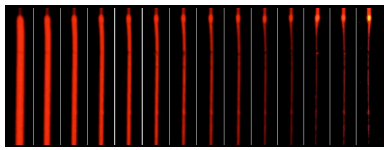


Figure1a: Microfluidic device & dimensions (µm), 1b: width of focused stream at constant fluid flow rate ratio and 1c: width of focused stream at changing fluid flow rate ratio (20-150)

The width of hydrodynamically focused lipid stream (w) is calculated by following equation:

$$\frac{w}{d} = \frac{1}{\sqrt{2\phi}} \qquad (1)$$

Where, d = diameter of channel and Φ = ratio of lipid to aqueous flow rates (FRR).
Stream width calculated theoretically with equation (1) agrees with the stream width calculated experimentally in figure 1 b and 1c.

Mechanism of liposome synthesis : In microfluidics, there is fine interface between lipid dissolved in alcohol and aqueous phase, and water and alcohol continuously diffuses along interface. Lasic D.D. proposed a theory of "bilayer phospholipid fragments (BPF) for liposome self assembly. According to this theory, when phospholipids dissolved into organic solvent, it transforms into the special intermediate structure called BPF due to change in solubility conditions (Lasic D. 1993). Hydrodynamic focusing of the lipid stream in aqueous stream reduces the solubility conditions of phospholipid; causing thermodynamic instabilities at the edges of sheet of PBFs. To avoid this interactions, bending and closing of BPFs causes self assemblies or vesicle formation.

Effect of flow rate ratios on liposome size : Figure 2a is DLS result of SPC-liposome at constant FRR 50. The flow rate of lipid/ethanol stream was increased from 1 to 8 µL/min and that of PBS from 50 to 400 µL/min, keeping FRR constant at 50. It is very much clear from figure 2a and 2b that the size and polydispersity of liposome depends on the flow rate ratio of two fluids. There is good correlation between fig 1b and 2a as former shows at constant FRR width of focused stream is constant and later shows size is also constant (233.4 +/- 35.26 nm) at the same flow conditions.

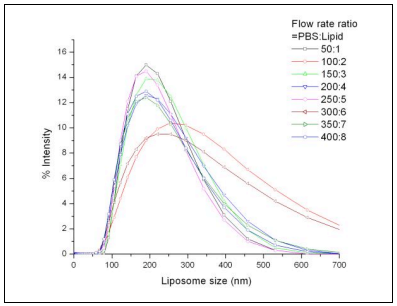


Figure 2a DLS/PCS: Intensity average size of liposome at constant flow rate ratio 50.

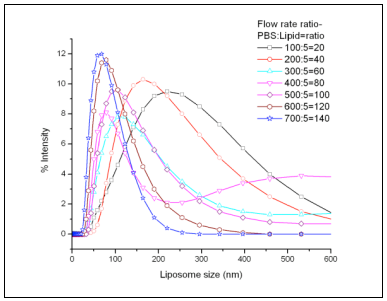


Figure 2b: Average size of liposome at changing flow rate ratio form 20 -140.

A same phenomenon is happening with figure 1c and 2b. In fig 2b, the lipid stream volumetric flow rate was kept constant at 5 µL/min and PBS volumetric flow rate was increased from 100 µL/min to 700 µL/min so as to increase FRR from 20 to 140. Here it is clear that as the FRR increases, the size and polydispersity decreases from 292 nm +/- 63.1 (for FRR20) to 64.58 nm +/- 10.61 (for FRR 140). Also it is very clear that there is no effect of shear rate on size of liposome as in case of constant FRR, size remains constant though shear rate increases considerably.

Transmission electron microscopy (TEM) : Figure 3a and 3b are the negative staining TEM images of FRR 80 and 140. Ammonium molybdate 2% solution was used as staining agent. It is

confirmed that at FRR 80, size (number average size 61.01 nm) and polydispersity of liposome is high compared to FRR 140 (number average size 39.36 nm)

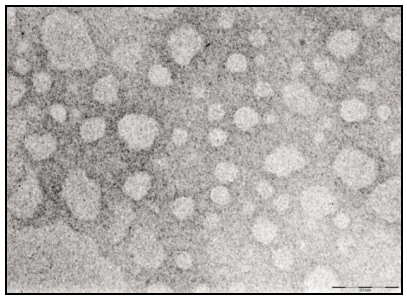


Figure 3a : TEM image showing larger and broader distribution (bar 100 nm)

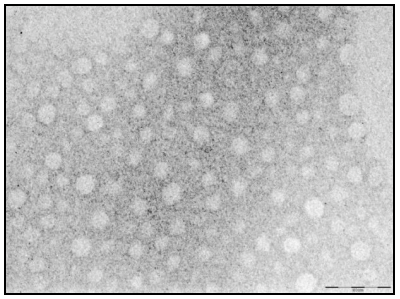


Figure 3b : TEM image showing smaller and monodispersed liposomes (bar 100 nm)

CONCLUSION

The microfluidic synthesis of liposome is one step method. The size ranging from 50 nm to 300 nm can be achieved by simply changing the flow rate ratios of lipid to aqueous phase. Lipophilic drug can be mixed in lipid solution in ethanol and hydrophilic drug in PBS buffer for encapsulation. Encapsulation is high compare to traditional method as no need for harsh steps (high pressure homogenization, extrusion, sonication etc.) for making monodispersed liposome population, where considerable loss of drug occurres. As the flow rate ratio of lipid to aqueous phase increased, the size and size distribution decreased. At constant ratio, liposome size and size distribution remained almost constant. The liposome size is depend on concentration of lipid as well as the ratio of lipid, alcohol and water, thus we can correlate the width of focused stream with size of liposome at particular lipid concentration. The formula derived for theoretical calculation of width of axially focused stream showed good agreement with experimental results.

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