UNEXPECTEDLY HIGH ENTRAPMENT EFFICIENCIES IN NANOMETER SCALE LIPOSOMES WITH HYDRODYNAMIC FOCUSING USING CONTINUOUS-FLOW MICROFLUIDICS

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ABSTRACT

A method is presented for achieving high entrapment efficiencies of a hydrophilic drug simulant, sulforhodamine B (SRB), in nanometer-scale liposomes using a continuous-flow microfluidic system. Liposome size and size dispersion are determined using tandem Asymmetric Flow Field Flow Fractionation (AF^4) and Multi-Angle Laser Light Scattering (MALLS). The average number of encapsulated SRB molecules in a single liposome is measured with Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cumulant Analysis (FCA). Our results show that this system allows for controlled loading of SRB into the liposomes with high entrapment efficiencies (EE) measured as a ratio of SRB concentration inside the liposome to SRB starting concentration in the hydration buffer.

KEYWORDS: liposomes, encapsulation, microfluidics, viscosity anisotropy

INTRODUCTION

The microfluidic continuous-flow injection method for the formation of homogeneous liposome distributions described previously is used to encapsulate SRB [1]. The controlled encapsulation of water-soluble compounds into the interior aqueous core of the liposomes is desirable for the use of liposomes as carriers for drug delivery and for contrast agent applications. The main objective is to achieve selective, and sufficiently high, localization of "active" drug or contrast agent at the disease site such as tumor or inflamed tissue. The use of liposomes as drug delivery systems can improve the pharmacological properties of many drugs resulting in increased circulation lifetimes and enhanced efficacy due to altered pharmacokinetic and biodistribution properties of the drug. Our work is also motivated by an economical standpoint as it is desirable to increase the entrapment efficiency and consequently reduce the waste of non-encapsulated compounds.

EXPERIMENTAL

A stream of lipids (2.5 mmol/L dimyristoylphosphatidylcholine, 2 mmol/L cholesterol, 0.5 mmol/L dicethylphosphate) dissolved in 2-propanol is hydrodynamically focused in a microfluidic channel and sheathed between two oblique phosphate buffered saline (PBS) (10 mmol/L phosphate, 2.7 mmol/L potassium chloride, 138 mmol/L sodium chloride, pH≈7.4, 3 mmol/L sodium azide) streams containing SRB, Figure 1A. The channels were fabricated in silicon and sealed with an anodically bonded borofloat glass cover. The water-soluble

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

fluorescent SRB dye is dissolved in the PBS at various concentrations from 10 µmol/L to 250 µmol/L.

The fluorescence of a sample of liposomes was measured with an inverted confocal microscope. The analyzed liposome and free dye samples were prepared by transferring 100 µL of solution into a well formed in a microscope slide. The detected photons were analyzed to create a photon counting histogram (PCH) and an autocorrelation function (ACF) for 10 s time intervals. For each sample 20 to 25 10 s intervals of data were collected. The autocorrelation of each liposome sample was used to extract the average number of background dye molecules, N_d, within the laser excitation volume. It is assumed that a liposome containing n SRB molecules will be n times brighter than a free SRB molecule at concentrations below the non-self-quenching regime of SRB. In this work we assume that all liposomes in a sample are equally bright so that the relative brightness is $J=\langle n^2\rangle/\langle n\rangle=\langle n\rangle$ ($\langle n\rangle$ is the average number of SRB molecules per liposome).

RESULTS AND DISCUSSION

The laminar flow in the microfluidic channel enables controlled diffusive mixing at the liquid-liquid interface. At a critical buffer-to-solvent ratio, the lipid monomers become insoluble and self-assemble into vesicles sequestering the surrounding fluid. The fine control of the buffer-to-solvent flow rate ratio ($FRR_{B/S}$) allows for facile and reproducible adjustment of the lipid self-assembly and the resultant liposome size and size dispersion as previously reported [1]. Figure 1B shows the size and size distribution for two different $FRRs_{B/S}$ that were chosen to study the encapsulation characteristics of 60 nm and 80 nm vesicles. Increasing the degree of hydrodynamic focusing results in smaller and more homogenous liposome populations.

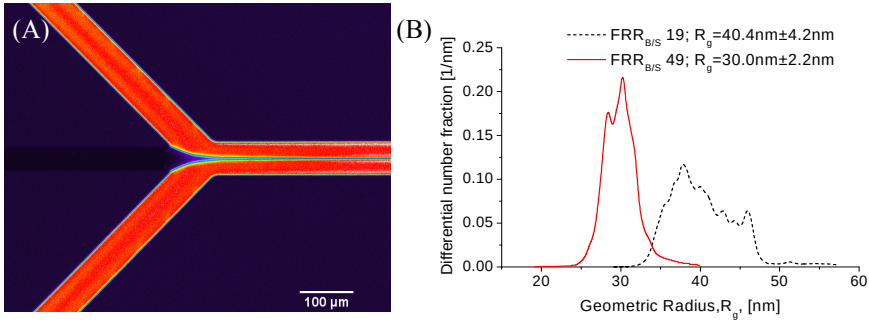

Figure 1 (A) Fluorescence micro-graph of the focusing section of the microfluidic network. SRB dissolved in PBS is injected through the slanted side channels and appears red. The lipid tincture is injected through the left center channel and contains no fluorescent dye. (B) Liposome size and size distribution at a $FRR_{B/S}$ of 19 and 49 and at a constant total volumetric flow rate of 100 µL/min. *As the $FRR_{B/S}$ increases from 19 to 49, the liposome mean geometric radius (R_g) and size distribution (one standard deviation) decreases from about 40* nm±*4.2* nm *to 30* nm±*2.2* nm.

The entrapment efficiency (EE) of SRB into liposomes was studied by injecting SRB dissolved in PBS at concentration from 10 µmol/L to 250 µmol/L. Preliminary results show unexpectedly high EE_{49} for the 60 nm diameter liposomes formed at a $FRR_{B/S}$ of 49. The higher EE, although not yet confirmed, may be due to a spatial SRB concentration enhancement induced by viscosity anisotropy in the microchannel that occurs when certain alcohols including 2-propanol mix with aqueous solutions. Table 1 shows the average number of encapsulated SRB molecules per liposome at different SRB concentrations in PBS. The number of encapsulated SRB molecules generally decreases as the SRB concentration decreases while the EE increases. This effect is more pronounced for the smaller liposomes which in addition to their reduced internal volume encapsulate more SRB molecules when the SRB concentration in the mobile phase decreases below 125 µmol/L. Liposomes with 60 nm diameter encapsulate comparable amounts of SRB while their internal volume is about two and a half times less than that of 80 nm diameter liposomes. At lower SRB concentrations they encapsulate even more SRB than the larger liposomes. Further investigations are currently underway to validate this phenomenon.

Table 1. Number (J ± 95 % CI) and entrapment efficiency (EE ± 95 % CI) of SRB dye molecules in small ($FRR_{B/S}$=49) and large ($FRR_{B/S}$=19) liposomes.

SRB [µmol/L]	J_{19}/J_{49}	EE_{19}/ EE_{49} [%]
250	10.6 ± 0.5 / 8.0 ± 0.36	36 ± 11 / 77 ± 17
125	6.9 ± 0.36 / 5.9 ± 0.28	47 ± 15 / 139 ± 31
62.5	5.4 ± 0.35 / 5.9 ± 0.28	56 ± 18/ 226 ± 51
30	3.5 ± 0.19 / 5.5 ± 0.26	82 ± 26 / 539 ± 121
10	1.4 ± 0.10 / 3.3 ± 0.19	110 ± 35 / 1148 ± 261

CONCLUSION

The formation of liposomes and encapsulation of a hydrophilic drug simulant (SRB) with high EE has been demonstrated using continuous-flow microfluidics. We hypothesize that viscous anisotropy as a result of the mixing between IPA and PBS leads to diffusive retardation at locally high viscosities at the diffusive liquid-liquid interface and therefore spatial concentration enhancement of SRB. This new technique could yield an improvement over the generally low EE of liposomes observed with passive loading methods.

ACKLNOWLEDGMENT

We thank David Ross from NIST (Gaithersburg) for useful discussions and John Mooreland from NIST (Boulder) for assistance in fabrication of the microchannels.

REFERENCES

[1] A. Jahn, W.N. Vreeland, D.L. DeVoe, L.E. Locascio, M. Gaitan., Microfluidic directed formation of liposomes of controlled size, Langmuir, 23 (11), pp. 6289-6293, (2007).