SANS Study of Self Assembled Unilamellar Vesicles – Dilution, Charge Density and Thermal History

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Spontaneously forming unilamellar vesicles (ULVs) are an attractive alternative to conventionally produced ULVs for controlled release applications due to their affordability, reproducibility and narrow size distribution1. Of interest is the structure and kinetics of a phospholipid mixture which contains dimyristoyl-phosphatidylcholine (DMPC), dimyristoyl-phosphatidylglycerol (DMPG) and dihexanoyl-phosphatidylcholine (DHPC), and that self-assembles into ULVs. At total lipid concentrations, C_{lp} < 2 wt.%, it has been reported that at low temperatures the system initially forms disk-like micelles, that then transforms into low-polydispersity, stable ULVs as the temperature is increased beyond 45 °C. [1,2] To understand the formation mechanism of ULVs, we investigated the effects of dilution, thermal history and charge density using small angle neutron scattering (SANS).

Phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL) and were mixed at a molar ratio of 50 (DMPC):15 (DHPC):2 (DMPG) in 99.9% D_2O. All samples were initially prepared to a C_{lp} of 10 wt.%. The concentrated samples (10 wt.%) were then further diluted to a lower C_{lp} (0.3 wt.%) using either progressive dilution (p.d.) or concentration shock (c.s.). The sample prepared by p.d. underwent the following dilution steps (i.e. 10-5-2-1-0.3 wt. %) at 4 °C, while the sample prepared by c.s. was diluted immediately to 0.3 wt.% at 4 °C. Each sample was then heated to 50 °C using one of two methods; slow annealing over several hours, or a temperature jump (T-jump).

Preliminary SANS data were obtained using the newly developed N5-SANS capability. Neutron wavelengths (λ) of 5.23, 4 and 2.37 Å were used to cover a range of scattering vectors, q, from 0.01 Å^{-1} < q < 0.3 Å^{-1}. Further, SANS studies were also conducted at the NG3-SANS located at NIST (National Institute of Standards and Technology) Center for Neutron Research (NCNR, Gaithersburg, MD)[3]. The SANS data was appropriately reduced and best fit using a single spherical shell model with a polydispersity, p, of ULV radii.

A comparison of spontaneously forming ULVs prepared using the p.d. and c.s. dilution methods, is shown in (Figure 1). The best fits to the data indicate that the samples prepared by the p.d. method are characterized by low polydispersity ULVs (p<0.2) - as can be verified by the oscillation along the scattering curve at q~ 0.03 Å^{-1}. This oscillation is absent in the SANS data of the c.s. sample, where a monotonic decay is observed, indicating the formation of high polydispersity ULVs (p>0.4) [2].

The effect of heating rate on ULV formation using the slow annealing and T-jump, was also investigated. The ULV systems under study have two charge densities, namely: [DMPG]/[DMPC] =0.8% and 1.5%. It is known that charged lipids deter the formation of multilamellar vesicles (MLVs) due to the Coulombic force, as was reported previously[4]. Figure 2 shows that the resultant ULV radii are larger in the case of slow annealing (i.e., 770 ± 70 Å and 134 ± 8 Å for [DMPG]/[DMPC] = 0.8% and 1.5%, respectively) compared to those ULV formed via the T-jump (R = 170 ± 10 Å and 90 ± 2 Å for [DMPG]/[DMPC] = 0.8% and 1.5%, respectively) [5]. This is most likely the result that the slow annealing method allows for the disk-like micelles to interact with each other over extended periods of time before folding unto themselves (i.e. forming larger size ULVs). Moreover, the data (Figure 2) also show that a higher charge density effectively reduces the interactions between disk-like micelles, resulting in smaller size ULVs.

In summary, SANS data unambiguously show how different dilution methods, heating rates and charge densities affect ULV polydispersity and size. This study not only provides important insights with regards to the structural transformation of the phospholipid mixtures, but also shows a different way of effectively manufacturing nano-size, stable, monodisperse ULVs.

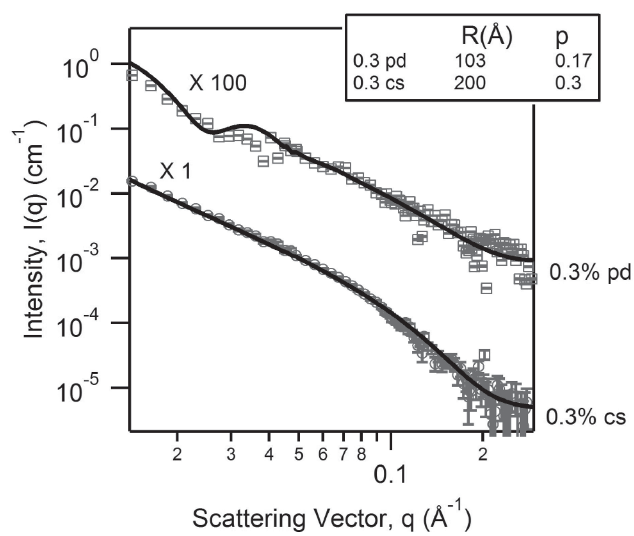

Fig. 1 TN5-SANS data (symbols) and best fits to the data (solid lines) of the two dilution methods [c.s. (circles) and p.d. (squares) at C_{lp} = 0.3 wt.% and 50°C] used to form ULVs. Higher polydispersity ULVs are found in the case of c.s.

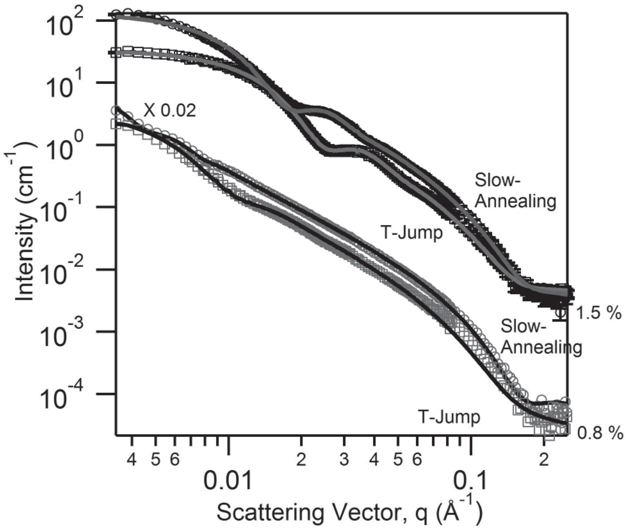

Fig. 2 *NG3 SANS data and best fits to the data (solid lines) comparing the T-jump (squares) and slow annealing (circles) methods to 50°C at C_{lp} = 0.3 wt.% with [DMPG]/[DMPC] = 0.8% (grey) and 1.5% (black). In both cases, the ULVs are found to be larger when they are formed using the slow annealing process.*

References

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