Supporting Information for

A Facile Route for Creating "Reverse" Vesicles: Insights into "Reverse" Self-Assembly in Organic Liquids

Shih-Huang Tung, Hee-Young Lee & *Srinivasa R. Raghavan**
Department of Chemical & Biomolecular Engineering
University of Maryland
College Park, MD 20742-2111
*Corresponding author. email: sraghava@eng.umd.edu

EXPERIMENTAL SECTION

Materials. Lecithin (95%) and C_4-lecithin (> 99%) were purchased from Avanti Polar Lipids. The C_4-lecithin was supplied as a solution in chloroform. Cyclohexane and NaCl were purchased from J. T. Baker. Deuterated cyclohexane (99.5%D) was purchased from Cambridge Isotopes. All chemicals were used as received

Sample Preparation. Mixed solutions containing short- and long-chain lecithin were prepared as follows. Lecithin and NaCl were dissolved in methanol to form 100 mM and 85 mM stock solutions, respectively. The desired amount of C_4-lecithin was dried from by evaporation in a vacuum oven for at least 12 h. Samples of desired composition were prepared by mixing the lecithin and NaCl stock solutions with the dried C_4-lecithin. Methanol was removed by evaporation in a vacuum oven for 48 h. The final samples with desired concentrations were obtained by adding cyclohexane or deuterated cyclohexane, followed by stirring at 60°C till the solutions became homogeneous. The samples were then sonicated by a water-bath type sonicator (Branson 1510) for 30 min.

DLS. A Photocor-FC light scattering instrument with a 5 mW laser light source at 633 nm was used at 25°C, with the scattering angle being 90°. A logarithmic correlator

was used to obtain the autocorrelation function, which was analyzed by the method of cumulants to yield a diffusion coefficient. The apparent hydrodynamic size was obtained from the diffusion coefficient through the Stokes-Einstein relationship.

SANS. SANS measurements were made on the NG-3 (30 m) beamline at NIST in Gaithersburg, MD. Neutrons with a wavelength of 6 Å were selected. The distances between sample chamber and detector were 1.35 m and 13.18 m. The range of scattering vector q was 0.004~0.4 $Å^{-1}$. Samples were prepared with deuterated cyclohexane and measured in 1 mm quartz cells at 25°C. The scattering spectra were corrected and placed on an absolute scale using calibration standards provided by NIST. The data are shown for the radially averaged intensity I versus the scattering vector $q = (4\pi/\lambda)\sin(\theta/2)$, where λ is the wavelength of incident neutrons and θ is the scattering angle. Modeling of SANS data was conducted using software modules provided by NIST to be used with the IGOR graphing package.[1]

SANS Modeling. For dilute solutions of non-interacting scatterers, the SANS intensity $I(q)$ can be modeled purely in terms of the form factor $P(q)$ of the scatterers (i.e., the structure factor $S(q) \rightarrow 1$ in this case). In this study, we consider form factor models for three different micellar shapes: spheres, rigid cylinders and unilamellar vesicles. In the expressions below, $\Delta\rho$ is the difference in scattering length density between the micelle and the solvent, so that $(\Delta\rho)^2$ is the scattering contrast.

Ellipsoids. The form factor $P(q)$ for ellipsoids of revolution with minor and major axes R_a and R_b is given by:[2,3]

$$P(q) = (\Delta\rho)^2 \left(\tfrac{4}{3}\pi R_a R_b^2\right)^2 \int_0^1 \left[3\frac{(\sin x - x\cos x)}{x^3}\right]^2 d\mu \qquad (1)$$

where $x = q\sqrt{\mu^2 R_b^2 + R_a^2(1-\mu^2)}$. Here μ is the cosine of the angle between the scattering vector q and the symmetry axis of the ellipsoid.

Rigid Cylinders. The form factor $P(q)$ for rigid cylindrical rods of radius R_c and length L is given by:[2,3]

$$P_{cylinder}(q) = (\Delta\rho)^2 \left(\pi R_c^{\,2} L\right)^2 \int_0^{\pi/2} \left[F(q,\alpha)\right]^2 \sin\alpha \, d\alpha \tag{2}$$

where

$$F(q,\alpha) = \frac{J_1\left(qR_c \sin\alpha\right)}{\left(qR_c \sin\alpha\right)} \cdot \frac{\sin\left(qL\cos\alpha/2\right)}{\left(qL\cos\alpha/2\right)} \tag{3}$$

Here α is the angle between the cylinder axis and the scattering vector q and J_1(x) is the first-order Bessel function of the first kind.

Unilamellar Vesicles. The form factor $P(q)$ for unilamellar vesicles of radius R and bilayer thickness t is given by the following expression:[2,3]

$$P(q) = (\Delta\rho)^2 \left\{ \frac{4}{3}\pi R^3 \frac{3J_1(qR)}{qR} - \frac{4}{3}\pi (R+t)^3 \frac{3J_1[q(R+t)]}{q(R+t)} \right\}^2 \tag{4}$$

$J_1(x)$ is the first-order Bessel function, given by:

$$J_1(x) = \frac{\sin x - x\cos x}{x^2} \tag{5}$$

For thin bilayers ($t << R$), or equivalently for large vesicles, $P(q)$ reduces to the following expression:

$$P(q) = (\Delta\rho)^2 \cdot (4\pi R)^2 \cdot \frac{t^2}{q^2} \sin^2(qR) \tag{6}$$

Eq (6) indicates that for large, non-interacting vesicles, $I(q)$ should show a q^{-2} decay in the low q range. If, the vesicles are polydisperse, the form factor has to be averaged over the vesicle distribution in the following manner:[2,3]

$$P(q) = \int f(R) \cdot P(q,R) \, dR \tag{7}$$

where $P(q, R)$ is the form factor for a vesicle of radius R (eq 6). The polydispersity in vesicle radius $f(R)$ can be accounted for by a Schultz distribution:

$$f(R) = \left(\frac{p+1}{R_0}\right)^{z+1} \frac{R^z}{\Gamma(z+1)} \exp\left(-(z+1)\frac{R}{R_0}\right) \tag{8}$$

In the above expression, R_0 is the average vesicle radius and Γ is gamma function. The polydispersity p_d is given by:

$$p_d = \frac{1}{\sqrt{z+1}} \tag{9}$$

TEM. TEM was conducted on a Jeol JEM 2100 microscope at 80 KeV. The positive staining agent, ammonium molybdate (from Sigma-Aldrich), was dissolved in methanol to form a 13 mM stock solution. Desired amounts of this compound were combined with the stock solutions during sample preparation, as described above. The final reverse vesicle samples were diluted to 1 mM and a 1 μL drop was applied on a carbon-coated copper grid, which was then air-dried before imaging was conducted.

REFERENCES

[1] Kline, S. R. "Reduction and analysis of SANS and USANS data using IGOR Pro." *Journal of Applied Crystallography* **2006**, *39*, 895-900.

[2] *Neutron, X-Ray and Light Scattering: Introduction to an Investigative Tool for Colloidal and Polymeric Systems*; Zemb, T.; Lindner, P., Eds.; Elsevier: Amsterdam, 1991.

[3] Pedersen, J. S. "Analysis of small-angle scattering data from colloids and polymer solutions: modeling and least-squares fitting." *Advances in Colloid and Interface Science* **1997**, *70*, 171-210.