

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

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Abstract: Reverse vesicles are spherical containers in organic liquids (oils) consisting of an oily core surrounded by a reverse bilayer. They are the organic counterparts to vesicles in aqueous solution and could potentially find analogous uses in encapsulation and controlled release. However, few examples of robust reverse vesicles have been reported, and general guidelines for their formation do not exist. We present a new route for forming stable unilamellar reverse vesicles in nonpolar organic liquids, such as cyclohexane and *n*-hexane. The recipe involves mixing short- and long-chain lipids (lecithins) with a trace of a salt such as sodium chloride. The ratio of short- to long-chain lecithin controls the type and size of self-assembled structure. As this ratio is increased, a spontaneous transition from reverse micelles to reverse vesicles occurs. Small-angle neutron scattering (SANS) and transmission electron microscopy (TEM) confirm the presence of unilamellar vesicles in the corresponding solutions. Average vesicle diameters can be tuned from 60 to 250 nm depending on the sample composition.

1. Introduction

The term “vesicle” refers to spherical containers formed in aqueous solution by the self-assembly of amphiphilic molecules.^{1,2} While the core of the vesicle contains water, its outer shell is composed of a bilayer of the amphiphiles, with these molecules oriented in such a way that their hydrophobic portions are inside the bilayer and thereby shielded from water (Figure 1). The amphiphiles that forms vesicles roughly have the shape of a cylinder, that is, their “critical packing parameter” is close to 1.¹ Such a shape can generally be achieved by two-tailed surfactants (lipids)² or by mixtures of cationic and anionic single-tailed surfactants.³ Vesicles have long held a fascination for scientists because of their structural resemblance to primitive biological cells. More importantly, vesicles are of technological interest for applications ranging from drug delivery and controlled release to bioseparations and sensing.² In particular, vesicles can encapsulate a variety of water-soluble solutes such as drugs, cosmetic ingredients, or agrochemicals in their aqueous core. These solutes can subsequently be released slowly and in a controlled manner through the vesicle bilayer.

Much like the above “normal” vesicles in water, one can also envision their counterparts in organic, nonpolar solvents (“oils”), and these are termed “reverse” vesicles.^{4–8} Such reverse vesicles

will have a “reverse” bilayer shell, where the hydrophobic portions of the precursor amphiphiles are exposed to the oily medium both in the core and in the exterior (Figure 1). Reverse vesicles are more rare than normal vesicles, but one might imagine that they too could find numerous applications much like normal vesicles, for example, for encapsulation and controlled delivery of hydrophobic solutes.^{4–8} A few attempts to assemble reverse vesicles have been reported, based on polyoxyethylene ethers,⁴ phospholipids,⁵ amino acid derivatives,⁶ sucrose esters,⁷ and metallosurfactants.⁸ However, questions persist about the stability, robustness, and ease of preparation of reverse vesicles by these methods, and as a result, these structures are not being widely exploited at the moment for applications. From a scientific standpoint, no clear rules or guidelines have been laid out for the systematic tuning of reverse aggregate geometry, in contrast to the extensive knowledge of the same for normal aggregates.

In this paper, we report a new route for forming stable unilamellar reverse vesicles in nonpolar organic liquids such as cyclohexane. Our method also offers a general framework for tuning reverse aggregate geometry from reverse spherical micelles to reverse cylindrical micelles and finally to reverse vesicles. The key ingredients in our samples are a combination of long- and short-chain phospholipids. The long-chain lipid is L- α -phosphatidylcholine (lecithin), a natural two-tailed lipid with an average tail length of 17 carbons and an unsaturation in one of the tails (Figure 1). The short-chain lipid we have used is 1,2-dibutyroyl-*sn*-glycero-3-phosphocholine (C₄-lecithin) with

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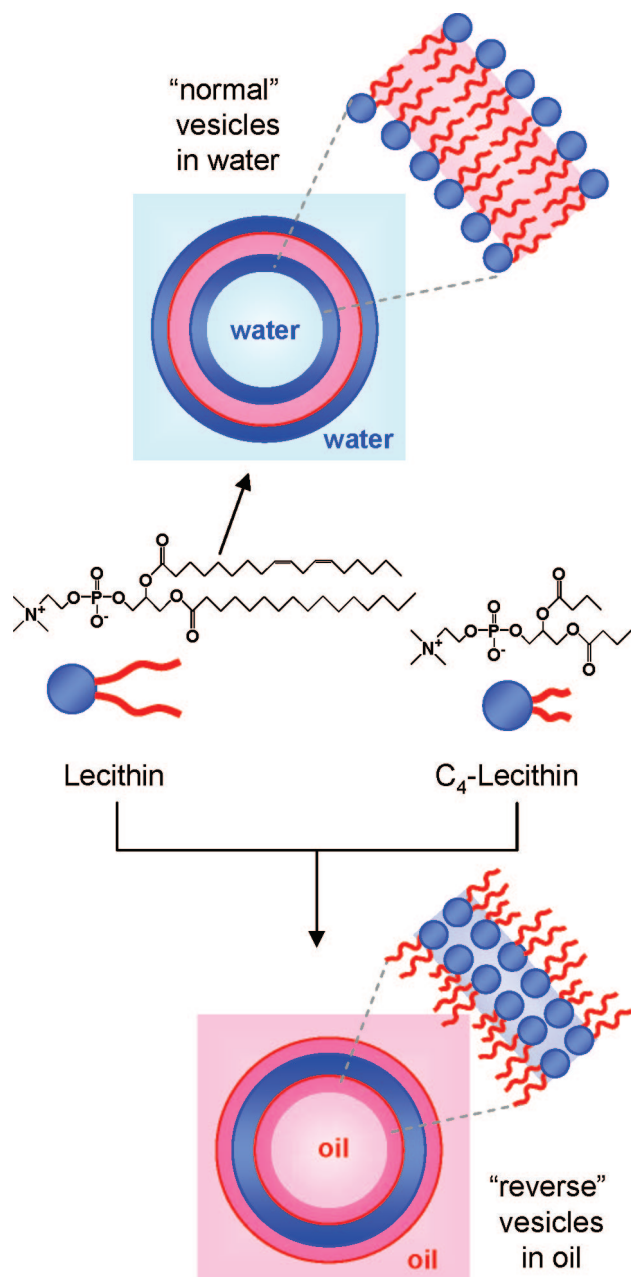


Figure 1. Molecular structures of lecithin (L- α -phosphatidylcholine) and C₄-lecithin (1,2-dibutyryl-*sn*-glycero-3-phosphocholine). Each molecule is schematically depicted as having a hydrophilic head (in blue) and two hydrophobic tails (in red). As is well-known, lecithin alone when added to water forms normal vesicles, which are illustrated schematically in the top panel (note here that the lipid heads are arranged on the outward and inward faces of the bilayer such that they are in contact with water). The present study shows that mixtures of lecithin and C₄-lecithin, when added to nonpolar solvents ("oils"), form reverse vesicles, as illustrated in the bottom panel. Note that the reverse vesicles have an oily core and the lipid tails are arranged on the outward and inward faces of the reverse bilayer.

two four-carbon saturated tails (Figure 1). Schematics of the two molecules, with blue heads and red tails, are also shown in Figure 1.

Let us examine what is known regarding the behavior of each of the above lipids, both in water and in oil. When added to water, lecithin alone forms large unstable multilamellar vesicles, while C₄-lecithin alone tends to form micelles.² Mixtures of short- and long-chain lecithins have also been studied in water,

and they assemble into disklike micelles (bicelles)⁹ or unilamellar vesicles.¹⁰ In oil, C₄-lecithin is insoluble, presumably because its tails are too short to compensate for the hydrophilic head. The longer-tailed lecithin, however, does dissolve in oil and assembles into reverse spherical micelles.^{11,12} Also, lecithin reverse spheres can be made to grow into reverse cylinders by adding trace quantities of water¹¹ or bile salts^{12,13} to the sample. If the reverse cylinders are long enough and flexible, they tend to entangle and thereby impart a strong viscoelastic character to the sample (such aggregates are then referred to as "reverse worms").¹²

Now, we consider a mixture of lecithin and C₄-lecithin in an organic liquid such as cyclohexane. The previously insoluble C₄-lecithin is able to dissolve in moderate amounts in the presence of the longer-tailed lecithin, and the resulting mixtures are homogeneous, single-phase solutions. As we will show, the type of aggregate in these mixtures can be tuned by varying the molar ratio of C₄-lecithin to lecithin. When this ratio is around 2, the solutions develop a bluish tinge and are found to contain reverse unilamellar vesicles. To ensure the stability of these reverse vesicles, it is critical that we also add a small amount of a salt, such as sodium chloride (NaCl), to the solution. (Again note that the salt is solubilized in cyclohexane only in the presence of the lipids.) In such salt-containing formulations, the reverse vesicles are very stable and robust, retaining their size and structure over a period of many months. As described below, we use dynamic light scattering (DLS), small-angle neutron scattering (SANS), and transmission electron microscopy (TEM) to study the reverse vesicles in these samples.

2. Results and Discussion

Phase Behavior. In cyclohexane, lecithin forms reverse spherical or ellipsoidal micelles and the resulting solutions are transparent and have a low viscosity essentially identical to that of the solvent.¹² To study the effect of adding C₄-lecithin, we conducted DLS at an overall lipid concentration of 20 mM in deuterated cyclohexane and as a function of the molar ratio R_0 of C₄-lecithin:lecithin. In addition to the two lipids, all the samples also contained 3.5 mM NaCl, the role of which is discussed below. Figure 2 shows the scattering intensity measured at 90° as a function of R_0 : the intensity increases dramatically at $R_0 \sim 0.7$ and then reaches a plateau. The strongly scattering samples had a clear, bluish tinge, as shown by the photograph of the $R_0 = 2.6$ sample in Figure 2. The bluish color is a manifestation of the Tyndall effect, indicating the presence of large scatterers in solution, and this is a general feature of vesicles in water as well.^{2,3} We should emphasize that the bluish samples still had a low viscosity, comparable to that of the solvent. The above data provide preliminary evidence for a phase transition from reverse micelles to vesicles with increasing R_0 . The average hydrodynamic diameter D_h of the reverse vesicles could be calculated from DLS, and it increased from 59 to 260 nm with increasing R_0 (Table 1). Further increase in R_0 beyond about 4.5 caused the samples to phase-separate into a turbid liquid phase and a solid precipitate. For the rest of this paper,

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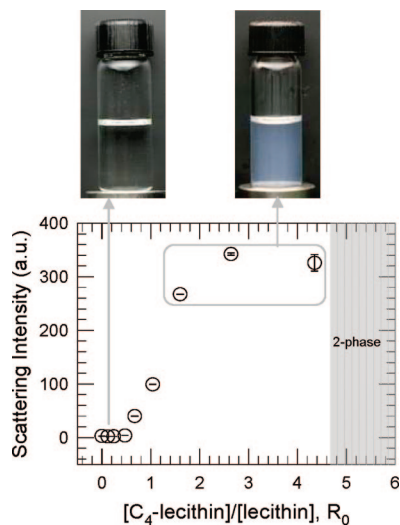


Figure 2. Light scattering intensity at 25 °C for lipid mixtures in deuterated cyclohexane as a function of R_0 , the molar ratio of C_4 -lecithin:lecithin. The total lipid concentration is held constant at 20 mM, and each sample additionally contains 3.5 mM NaCl. Photographs of two samples at different R_0 values are also shown. At low R_0 , the samples are transparent and colorless with a low scattering intensity. At an R_0 around 2.5, the scattering intensity of the sample is a factor of 10^2 higher and the sample shows a bluish hue, indicating strong light scattering from the vesicles (Tyndall effect). Finally, when R_0 exceeds ca. 4.5, the samples phase-separate into one liquid phase and one solid phase.

Table 1. Hydrodynamic Diameters at 25 °C from DLS^a

R_0	0.7	1.0	1.6	2.6
D_h (nm)	58.8 ± 0.8	79.0 ± 0.7	137.6 ± 0.8	260.3 ± 2.5

^a Samples containing C_4 -lecithin + lecithin in deuterated cyclohexane (20 mM total lipids; 3.5 mM NaCl). Data are shown for various values of R_0 , the molar ratio of C_4 -lecithin:lecithin.

the focus is on the stable reverse vesicle samples at moderate R_0 values between 2 and 4.

As mentioned above, the addition of an appropriate amount of a salt, such as NaCl, is necessary to stabilize the reverse vesicles. Otherwise, the vesicle samples are bluish and homogeneous initially, but within a few hours they tend to phase-separate into coexisting liquid phases. The maximum amount of salt that can be solubilized depends on the overall lipid concentration, suggesting that the salt is incorporated within the lipid assemblies. For example, at an overall lipid content of 20 mM, less than 8 mM NaCl can be incorporated. If too low an amount of salt is used (e.g., <2 mM of NaCl for a sample with 20 mM total lipid and a lipid molar ratio $R_0 = 2.6$), the vesicles are not stable. However, with a sufficient amount of salt, the reverse vesicles remain indefinitely stable and their size also remains unchanged with time.

Small-Angle Neutron Scattering. To further elucidate the microstructures in these samples, we resorted to SANS. For these experiments, samples were made in deuterated cyclohexane to achieve the needed contrast between scatterers and solvent. SANS spectra (I vs q) for 20 mM lipid solutions containing 3.5 mM NaCl are shown in Figure 3 at varying R_0 . The data for $R_0 = 0$ and 0.5 both asymptote to a plateau at low q and essentially correspond to micelles. In contrast, there is no plateau at $R_0 = 2.6$, with the data showing a q^{-2} decay of the intensity at low q . Such a decay is a signature of scattering

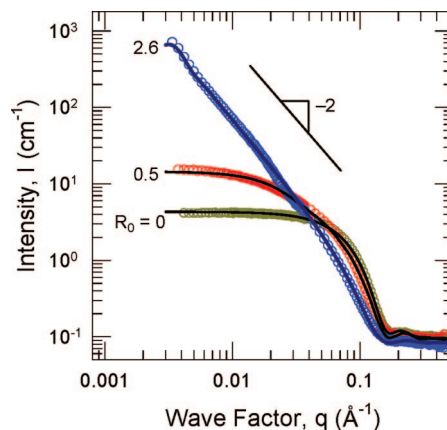


Figure 3. SANS data at 25 °C from lipid mixtures in deuterated cyclohexane for different C_4 -lecithin:lecithin molar ratios R_0 . The samples contain 20 mM total lipid and 3.5 mM NaCl. The solid curves through the data are fits to appropriate models (see text for details).

from vesicle bilayers (eq 6 in Supporting Information).^{14,15} Importantly, the q^{-2} decay is observed only for samples within the reverse vesicle region in Figure 2 (i.e., the region of bluish, low-viscosity samples). Thus, the SANS data support our hypothesis of a phase transition from reverse micelles to reverse vesicles with increasing R_0 .

To obtain a more quantitative picture of reverse micellar and vesicular sizes, we model the SANS data using appropriate form factors (eqs 1–9 in Supporting Information). The fits are shown as solid curves through the data in Figure 3. First, for the case of lecithin in cyclohexane ($R_0 = 0$), the reverse micelles are modeled as ellipsoids of revolution (eq 1 in Supporting Information), and we obtain radii of 2.2 and 3.0 nm, respectively, for their minor and major axes. Upon the addition of low amounts of C_4 -lecithin, the micelles grow axially, and for $R_0 = 0.5$, they can be modeled as rigid cylinders (eqs 2 and 3 in Supporting Information). The cylinder radius is found to be 2.2 nm (same as above) while their contour length is about 19.8 nm. At higher R_0 , the data are fit to the polydisperse unilamellar vesicle model (eqs 4–9 in Supporting Information). For $R_0 = 2.6$, an average reverse vesicle diameter of 234 nm is obtained, along with a bilayer thickness of ca. 3.7 nm, and a polydispersity of 0.22. This value of the average vesicle diameter is reasonably consistent with the hydrodynamic diameter obtained from DLS (Table 1). In sum, the SANS data confirm the evolution of self-assembled structures from reverse spherical micelles to reverse cylindrical micelles to reverse vesicles in mixtures of C_4 -lecithin and lecithin.

Transmission Electron Microscopy. Figure 4 shows TEM micrographs of the sample with $R_0 = 2.6$, which has been shown to contain reverse vesicles by DLS, SANS, and visual observations. Here, 0.8 mM ammonium molybdate was added as a positive stain that would bind with the headgroups of lipids and thus clearly reveal the bilayers. The TEM images show a number of spherical structures with distinct shells, much like conventional micrographs of unilamellar vesicles. The diameters of these structures range from less than 100 nm to 1 μ m. Note that many of these sizes are larger than those obtained from SANS and DLS. We believe this disagreement is due to the

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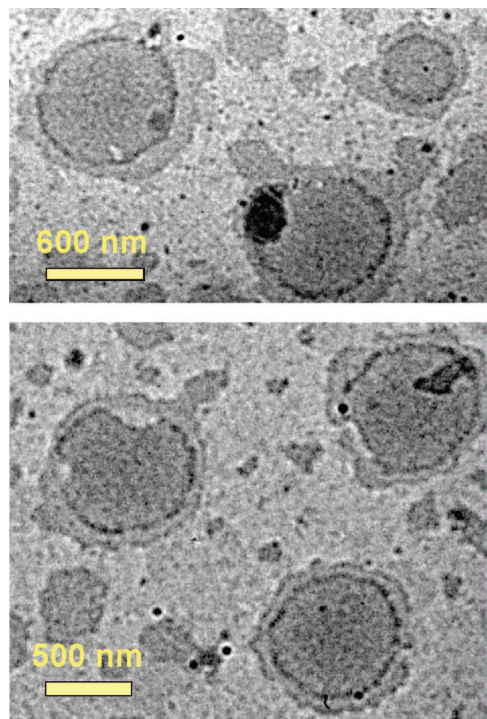


Figure 4. TEM image of structures present in a C_4 -lecithin + lecithin sample in deuterated cyclohexane at $R_0 = 2.6$ (total lipids = 20 mM, $[NaCl] = 3.5$ mM).

collapse of the reverse vesicles as the solvent, cyclohexane, is evaporated from the TEM grid. Also, during the evaporation process, the vesicles may come into contact by diffusion, and possibly fuse. Generally, conventional TEM (with or without staining) is not as reliable as the technique of cryo-TEM in deducing the structures present in solutions.¹⁶ However, the sample preparation steps in cryo-TEM are optimized only for aqueous solutions,¹⁶ and we have therefore not been able to successfully use this technique for our samples. Despite the limitations of conventional TEM, we stress out that our results using this technique are quite consistent and reproducible. Importantly, the spherical shell-like structures indicated in Figure 4 are found only in the reverse vesicle samples; they were not observed in the control (reverse micellar) samples.

Mechanism. We will now address the mechanism by which C_4 -lecithin transforms lecithin reverse micelles to reverse vesicles. Additionally, we will address the role of NaCl in this process. It is known that the shape of self-assembled structures formed by amphiphiles is governed by their geometry. This connection is usually expressed in terms of the critical packing parameter $p = a_{tail}/a_{hg}$, where a_{tail} and a_{hg} are the cross-sectional areas of the amphiphile's tail and headgroup, respectively.¹ In water, ionic surfactants in the absence of salt have a p around $1/3$ (i.e., a cone shape) and thus form spherical micelles. When salt is added, the effective headgroup area is reduced due to a decrease in the electrostatic screening length.^{1,3} The packing parameter p then increases to around $1/2$ (truncated cone shape) and the micelles, in turn, transform from spheres to cylinders. A further increase of p to around 1 (cylinder shape) leads to the formation of bilayers and vesicles.^{1,3} Lipids form vesicles in water because their two hydrophobic tails gives them a packing parameter close to 1.

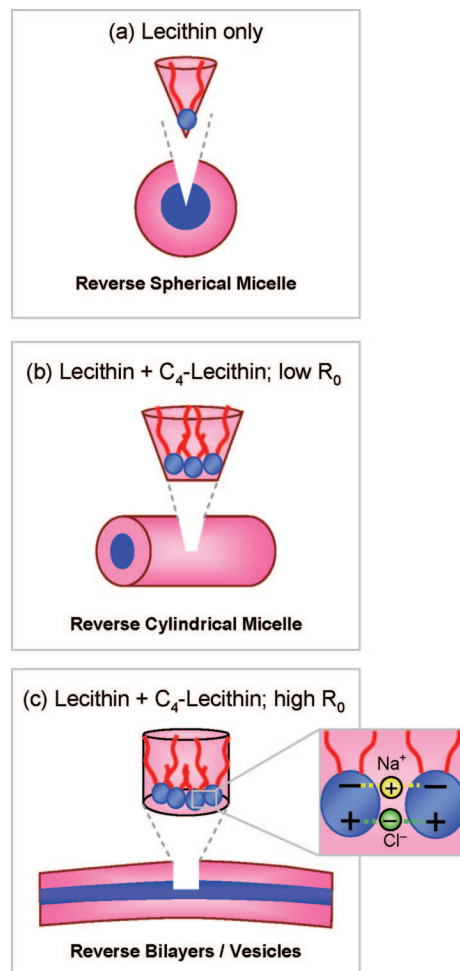


Figure 5. Schematics of the reverse self-assembled structures found in mixtures of lecithin and C_4 -lecithin in organic solvents (oils). (a) Lecithin alone tends to form spherical reverse micelles. (b) When C_4 -lecithin is added at low amounts, the headgroup area expands while the tail area remains about the same. The net molecular geometry is thus altered from a cone to a truncated cone, leading to the formation of cylindrical reverse micelles. (c) Further addition of C_4 -lecithin additionally expands the head area such that the molecular geometry becomes close to a cylinder. This induces the micelles to transform into reverse bilayers or vesicles. The inset shows that the reverse vesicles are stabilized by electrostatic interactions between the headgroups of C_4 -lecithin and lecithin through the mediation of salt (Na^+ and Cl^-) ions.

In the case of organic solvents, the shapes of self-assembled structures are still regulated by p but in an opposite way.¹² The formation of reverse micelles requires a packing parameter p much larger than 1, and spherical reverse micelles evidently correspond to an inverse cone shape (Figure 5a). For these spheres to transform into cylinders or vesicles, the packing parameter p has to decrease. Such a decrease can be caused, for instance, by the binding of C_4 -lecithin to the lecithin headgroups. That is, since C_4 -lecithin has the same headgroup as lecithin but much shorter tails, it increases the headgroup area a_{hg} while maintaining about the same tail area (note in Figure 5a that the tails are all directed outward into the organic solvent). The net effect is to decrease p , and at low values of R_0 (around 1, cf. Figure 2), the effective geometry resembles an inverse truncated cone so that the reverse spheres are induced to grow into reverse cylinders (Figure 5b). What happens as the C_4 -lecithin fraction, and therefore R_0 , is increased? Presumably, p can further decrease to a value closer to 1 (cylinder

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shape), and we believe this would induce a second transition—from reverse cylindrical micelles to reverse bilayers or vesicles (Figure 5c). The data show that such a transition to reverse vesicles occurs over moderate values of R_0 (around 2–4, cf. Figure 2), while phase separation ensues at higher R_0 .

We can thus rationalize reverse vesicle formation on the basis of molecular geometry. What then is the need for NaCl? To understand this, we need to delve into the driving forces for reverse self-assembly. For the case of "normal" self-assembly (in water), the dominant driving force is generally believed to be the hydrophobic interaction,¹ with electrostatic interactions between the headgroups being a secondary factor in some cases (e.g., in mixtures of cationic and anionic surfactants).² In organic solvents, hydrogen bonding has been suggested to be the driving force for the growth of reverse spheres to cylinders in lecithin/water and lecithin/bile-salt systems.^{11,12} However, C₄-lecithin and lecithin have the same zwitterionic headgroups, and thus it is unlikely that hydrogen bonding would play a role in the coassembly of their headgroups. In this regard, it is interesting that solutions containing only C₄-lecithin and lecithin become unstable and phase-separate. Presumably, the interactions between the two lipids are quite weak and thus insufficient to stabilize their mixed aggregates. The ability of NaCl to impart stability suggests that its role is to mediate the interactions between the lipids. Specifically, we hypothesize that the Na⁺ and Cl⁻ ions bind with the negative and positive charges on the lipid headgroups through short-range electrostatic interactions and thereby serve as a bridge between the C₄-lecithin and lecithin (Figure 5c, inset). Note that while salt–lipid interactions (especially for metal salts) have been widely studied in aqueous solution,^{17,18} such interactions in oils have not been investigated

due to the low solubility of salts in nonpolar media. Also, electrostatic interactions do indeed occur in nonpolar media,¹⁹ although their importance remains underappreciated. Further studies are required to clarify the nature of salt–lipid interactions and their connection with reverse vesicle stability.

3. Conclusions

In conclusion, we have demonstrated a new approach for creating reverse unilamellar vesicles that involves mixing short- and long-chain lecithins in cyclohexane along with a small amount of a salt such as NaCl. The presence of reverse vesicles in these solutions has been verified by DLS, SANS, and TEM, and the structures have been shown to be unilamellar, with diameters ranging from 60 to 300 nm. Moreover, our preparation technique provides a general framework for systematically tuning reverse aggregate morphology from reverse spheres to cylinders to vesicles, simply by controlling the molar ratio of the two lipids. We suggest that electrostatic interactions may be important in reverse vesicle formation and stabilization; in particular, NaCl is believed to act like a "glue" in binding lipid headgroups together and in stabilizing the reverse vesicles in solution.

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Supporting Information Available: Detailed experimental section. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Supporting Information for

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EXPERIMENTAL SECTION

Materials. Lecithin (95%) and C₄-lecithin (> 99%) were purchased from Avanti Polar Lipids. The C₄-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 C₄-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 C₄-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}

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

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}

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

where

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

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}

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

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

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

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

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

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}

$$P(q) = \int f(R) \cdot P(q, R) dR \quad (7)$$

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:

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

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

$$p_d = \frac{1}{\sqrt{z+1}} \quad (9)$$

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 μ L drop was applied on a carbon-coated copper grid, which was then air-dried before imaging was conducted.

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