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Can Simple Salts Influence Self-Assembly in Oil? Multivalent Cations as Efficient Gelators of Lecithin Organosols

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It is known that lecithin, a zwitterionic phospholipid, self-assembles into spherical reverse micelles in organic solvents. We have explored the effects of adding inorganic salts to lecithin organosols. Salts are insoluble in organic solvents, and hence their effects on reverse self-assembly have rarely been studied. Our studies show, however, that salts can indeed be dissolved in organic liquids in the presence of lecithin. More interestingly, salts of multivalent cations like calcium (Ca^{2+}), magnesium (Mg^{2+}), lanthanum (La^{3+}), and cerium (Ce^{3+}) greatly increase the viscosity of lecithin organosols and transform the samples into optically transparent organogels. In comparison, monovalent cations or transition-metal cations have negligible effect on reverse self-assembly. On the basis of data from small-angle neutron scattering (SANS), we show that gelation is accompanied by a nanostructural transition from spherical micelles to cylindrical micelles/filaments. The varying abilities of different cations to induce gelation is shown to correlate with their binding tendencies to the phosphocholine headgroups of lecithin. A two-component gelator such as lecithin/Ca^{2+} could be attractive for applications due to its negligible cost and nontoxic nature. We demonstrate how such a gelator combination can convert a liquid fuel such as kerosene into a gel without the use of heat or shear. The same gel can also further be ungelled by addition of a few drops of alcohol.

1. Introduction

It is well-known that amphiphilic molecules can self-assemble in water into characteristic structures such as micelles and vesicles.[1,2] Aqueous self-assembly of amphiphiles is recognized to be driven primarily by hydrophobic interactions, but electrostatic interactions are known to play an important role as well. For example, take the effect of added salt (e.g., NaCl or $CaCl_2$) on a cationic surfactant in water with respect to either the critical micelle concentration (CMC) of the surfactant or the size and shape of its micelles. It is known that salts reduce the CMC and that they can induce a transition from spherical to long cylindrical micelles (also called wormlike micelles or worms[3]). Such effects are easily rationalized in terms of classical electrostatic theory:[1,2] the salt ions reduce the electrostatic repulsions between the cationic surfactant headgroups (in effect decreasing the Debye screening length), which facilitates micellization and assembly into cylindrical structures. Salt effects on aqueous self-assembly thus have a consistent physical basis.

Self-assembly of amphiphilies can also occur in nonpolar organic liquids ("oils" for short) and this is referred to as "reverse" self-assembly.[1,2] Compared to self-assembly in water, much less is known about reverse self-assembly;[4−6] for instance, amphiphiles in oil may or may not have a well-defined CMC. Phase diagrams for amphiphiles in organic solvents have also not been mapped out in detail as they have for water. An additional issue that has rarely been considered is the effect of adding salt to a solution (organosol) of an amphiphile in oil. Will the added salt influence the assembly process? At first, this question may seem nonsensical because practical experience teaches us that salts like NaCl and $CaCl_2$ cannot be dissolved in oil. However, in reality, small amounts of salts *can* be dissolved in oil when an amphiphile is also present. This is akin to solubilizing small amounts of oil in water containing surfactant micelles (the resulting mixture is then termed a microemulsion).[2] In fact, in a similar vein, hydrophilic molecules such as enzymes have previously been solubilized in oil within the interior of reverse micelles.[7,8] If salts can indeed be introduced into oil, the interesting question then is whether the *type* of salt (e.g., NaCl vs $CaCl_2$) would matter. The present study will show a class of oil-based systems in which the type of salt critically impacts self-assembly.

The systems reported in this paper are solutions in nonpolar organic liquids of the two-tailed phospholipid, lecithin along with a simple inorganic salt. Lecithin is well-known to form reverse spherical or ellipsoidal micelles in a range of organic liquids.[5,9] Previous research has also shown that the addition of water in small amounts to a lecithin organosol can induce the growth of reverse micelles from spheres to long, flexible cylindrical micelles (worms).[9,10] More recently, we have reported that the same effect can also be induced by the addition of bile salts, which are a class of naturally occurring steroidal amphiphiles.[11,12] These bile salts were insoluble if directly added to oils, but could be solubilized in the presence of lecithin.[11,12] In the present study, we shifted our

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attention from bile salts to more common, inorganic salts. We find that the latter can also be solubilized in lecithin organosols and, moreover, the type and concentration of salt control self-assembly in these mixtures.

The most interesting result from our study is the *strong specificity of the salt cation*: in particular, divalent cations from group 2 of the Periodic Table (specifically Ca^{2+} and Mg^{2+}) and trivalent lanthanides (La^{3+} and Ce^{3+}) convert lecithin organosols into optically transparent organogels. This macroscopic change in sample rheology is driven by a transition from spherical reverse micelles to cylindrical micelles/filaments,[9,11] as we shall show using small-angle neutron scattering (SANS). In comparison, monovalent cations from group 1 of the Periodic Table (e.g., Na^+ and K^+) have negligible influence on self-assembly. Also, transition metal cations, both divalent (e.g., Co^{2+}, Cu^{2+}, and Cd^{2+}) and trivalent (e.g., Fe^{3+}) have no effect. The striking results we observe with divalent cations and lanthanides are consistent with their strong propensity to bind with the phosphocholine headgroups of lecithin.[13−18] Lipid−ion interactions have been well-studied in aqueous media and we will use those findings in putting together a mechanism for the multivalent cation-induced transformation of lecithin reverse micelles.

Apart from its fundamental implications, the formation of organogels by combining lecithin and a Ca^{2+} (or similar) salt may also be of technological interest. Weiss and co-workers[19,20] have emphasized the search for the "simplest types" of organogelators. While a few simple gelators have been found (e.g., certain long-chain *n*-alkanes[20]), most current molecular gelators are complex organic structures that need to be synthesized in the laboratory.[19−21] In comparison, the lecithin/Ca^{2+} combination may be among the simplest and least expensive options for organogelation. Note that lecithin is a widely available, low-cost, food-grade material. Lecithin/Ca^{2+} gels are likely to be biocompatible and nontoxic; they could even be used safely to make homemade gels out of edible oils. Gels can be formed with organic fuels such as kerosene and gasoline and at less than 4 wt % overall of the additives. We have also been able to make lecithin/Ca^{2+} organogels isothermally, i.e., without the use of heat, and also without employing any mixing or shear (see Figure 6 later). These gels are indefinitely stable at room temperature, but can be converted to sols by heat or by addition of a polar cosolvent. Lecithin/salt mixtures are thus a class of organogelators with much practical potential.

2. Experimental Section

Materials. Soybean lecithin (95% purity) was purchased from Avanti Polar Lipids. The following anhydrous (> 99.99% purity) salts were purchased from Sigma-Aldrich: $MgCl_2$, $CaCl_2$, $CaBr_2$, CaI_2, $SrCl_2$, $BaCl_2$, $LaCl_3$, and $CeCl_3$. All other salts were purchased from Sigma-Aldrich, J. T Baker, or Fisher-Scientific. Organic solvents (at least >99% purity) were purchased from Sigma-Aldrich or Fisher Scientific.

Sample Preparation. Mixed solutions containing lecithin and salts were prepared as follows. Lecithin and salt were separately dissolved in methanol (or in some cases, ethanol) to form 100 mM stock solutions. Samples of particular compositions were prepared by mixing the stock solutions. The solvent was removed by drying the samples under a fume hood for 24 h and then in a vacuum oven for at least 48 h. The final samples were obtained by adding the organic solvent, followed by stirring and heating at 60 °C until the solutions became transparent and homogeneous. The above procedure ensured the removal of any residual water from the sample, and thereby facilitated reproducible sample preparation. An alternate procedure that does not involve any heating or stirring is described in the Results section (see Figure 6).

Rheological Studies. Steady and dynamic rheological experiments were performed on an AR2000 stress controlled rheometer (TA Instruments). Samples were run on cone-and plate geometries (20 mm dia/2° cone-angle or 40 mm dia/4° cone-angle). The plates were equipped with Peltier-based temperature control and all samples were studied at 25 ± 0.1 °C. A solvent trap was used to minimize evaporation of solvent. Dynamic frequency spectra were conducted in the linear viscoelastic regime of the samples, as determined from dynamic strain sweep measurements. For steady-shear experiments, sufficient time was allowed before data collection at each shear rate to ensure that the viscosity reached its steady-state value.

Small Angle Neutron Scattering (SANS). SANS measurements were made on the NG-7 and NG-3 (30 m) beamlines at NIST in Gaithersburg, MD. Neutrons with a wavelength of 6 Å were selected. Three sample−detector distances were used to obtain data over a range of wave vectors from 0.004 to 0.4 $Å^{-1}$. Samples were studied in 1 mm or 2 mm quartz cells at 25 °C. Scattering spectra were corrected and placed on an absolute scale using NIST calibration standards. The data are shown as plots of the absolute intensity I versus the wave vector $q = 4\pi \sin(\theta/2)/\lambda$, 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.[22]

SANS Modeling. For dilute solutions of noninteracting scatterers, the SANS intensity $I(q)$ can be modeled purely in terms of the form factor $P(q)$ of the scatterers. In this study, we consider form factor models for ellipsoids and cylinders. In the expressions below, $\Delta\rho$ is the difference in scattering length density between the aggregate 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[23]

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

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

Cylinders. The form factor $P(q)$ for cylinders of radius R_c and length L is given by[23]

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

where

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

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. If the cylinders are polydisperse, the form factor has to be averaged over the length distribution in the following manner:

$$P(q) = \int f(L)\, P(q,L)\, \mathrm{d}L \quad (4)$$

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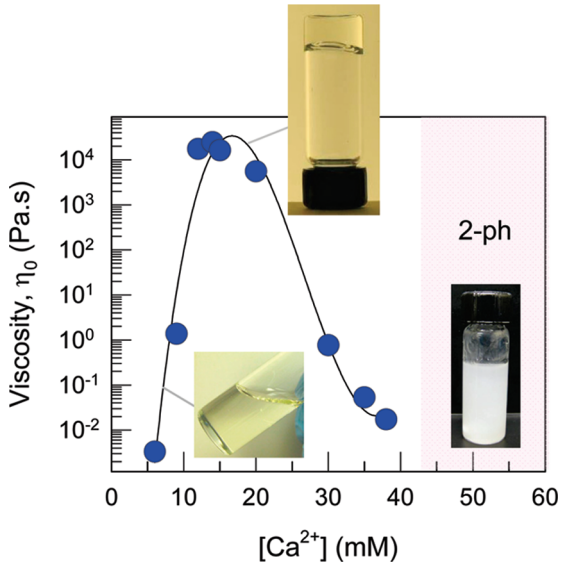

Figure 1. Zero shear viscosity η_0 of solutions in *n*-decane containing 40 mM lecithin and varying amounts of [Ca^{2+}]. Photographs of three samples at different [Ca^{2+}] are also shown. At low [Ca^{2+}], the solutions have a low viscosity. Around 14 mM [Ca^{2+}], the viscosity peaks and the sample is gel-like: it flows very slowly in the inverted vial. Beyond the peak, the viscosity drops precipitously, and around 40 mM [Ca^{2+}] the sample becomes turbid, indicative of phase-separation.

where $P(q, L)$ is the form factor for a cylinder of length L (eq 2). The polydispersity in cylinder length $f(L)$ can be accounted for by a Schultz distribution:

$$f(L) = \left(\frac{p+1}{L_0}\right)^{z+1} \frac{L^z}{\Gamma(z+1)} \exp\left(-(z+1)\frac{L}{L_0}\right) \qquad (5)$$

In the above expression, L_0 is the average cylinder length and Γ is the gamma function. The polydispersity index p_d is given by:

$$p_d = \frac{1}{\sqrt{z+1}} \qquad (6)$$

3. Results and Discussion

Phase Behavior and Rheology of the Gels. We first present results for mixtures of lecithin and anhydrous calcium chloride ($CaCl_2$) in *n*-decane. Figure 1 shows the zero-shear viscosity η_0 of solutions containing 40 mM of lecithin and various Ca^{2+} concentrations. At a [Ca^{2+}] of 5 mM or lower, the viscosity is close to that of the solvent. At slightly higher [Ca^{2+}], the viscosity rapidly increases. Samples with about 12−15 mM of Ca^{2+} are gel-like and their viscosities are about ten million times higher than that of the solvent. The photograph of such a sample is shown in the figure and one can see that the sample flows very slowly in the inverted vial, indicating gel-like behavior.[24] Note also that this organogel is optically clear and colorless; also there is no indication of any solid precipitate; that is, the salt is completely dissolved in the sample. The other interesting result from Figure 1 is that the viscosity shows a nonmonotonic behavior; it peaks around 14 mM Ca^{2+} and then falls sharply. By about 40 mM Ca^{2+}, the sample has reverted to a thin, clear liquid with a viscosity close to that of the solvent. Thereafter, around 43 mM Ca^{2+}, the sample becomes turbid while staying homogeneous. The turbidity is indicative of large structures and/or phase separation. Samples in this regime were not studied further.

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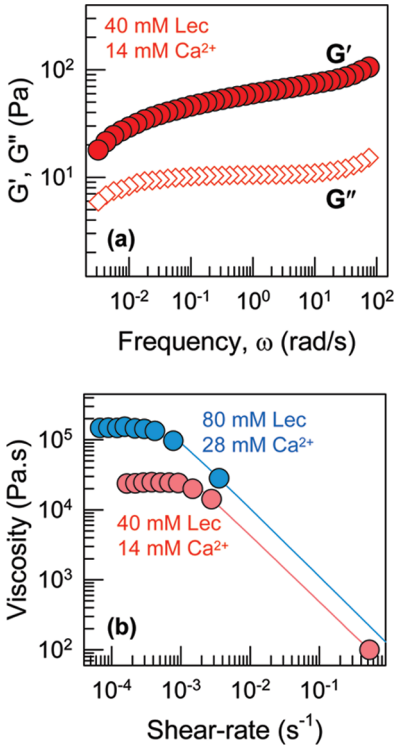

Figure 2. Dynamic and steady-shear rheology at 25 °C of gel-like lecithin−Ca^{2+} samples in *n*-decane. In (a), dynamic data (elastic modulus G' and viscous modulus G'' as functions of frequency ω) are shown for a sample of 40 mM lecithin +14 mM Ca^{2+}. In (b) steady-shear data (viscosity vs shear-rate) are shown for the above sample as well as for a sample containing 80 mM lecithin +28 mM Ca^{2+}.

Next, we used rheological techniques to characterize the gel-like lecithin/Ca^{2+} samples at intermediate [Ca^{2+}]. Figure 2a shows the dynamic rheological response (elastic G' and viscous G'' moduli as functions of frequency ω) of a sample containing 40 mM lecithin and 14 mM Ca^{2+}, which corresponds to the viscosity peak in Figure 1. Both moduli show a weak frequency dependence, with G' dominating over G'' across the entire frequency range; thus, the sample response is mostly elastic. The gap between the moduli narrows at low frequencies, but the curves do not intersect; this indicates that the relaxation time of the sample is very high and falls outside the window of time scales probed by rheometry.[25] The above response can be characterized as gel-like. A true gel would show frequency-independent moduli over the entire frequency range, reflecting infinite values of relaxation time and viscosity.[24,25] Here, the moduli have a weak frequency dependence and so the sample does relax, albeit very slowly. In turn, the sample has a finite but high viscosity, as can be seen from its steady-shear response (Figure 2b), which reveals a plateau in the apparent viscosity at low shear-rates. The plateau value is the zero-shear viscosity η_0 and it is the value plotted in Figure 1. All lecithin/Ca^{2+} samples we studied had finite η_0 values. Increasing the gelator concentration increased the value of η_0 but did not qualitatively change the nature of the rheological response. An example is shown in Figure 2b for a sample with 80 mM lecithin and 28 mM Ca^{2+}, which has a η_0 of about 10^5 Pa s.

The above data show that lecithin/Ca^{2+} mixtures can convert organic liquids like *n*-decane into clear, gel-like samples (we will refer to these samples as "organogels" for convenience). A lecithin:Ca^{2+} molar ratio of 40:14 or about 3:1 seems to be optimal

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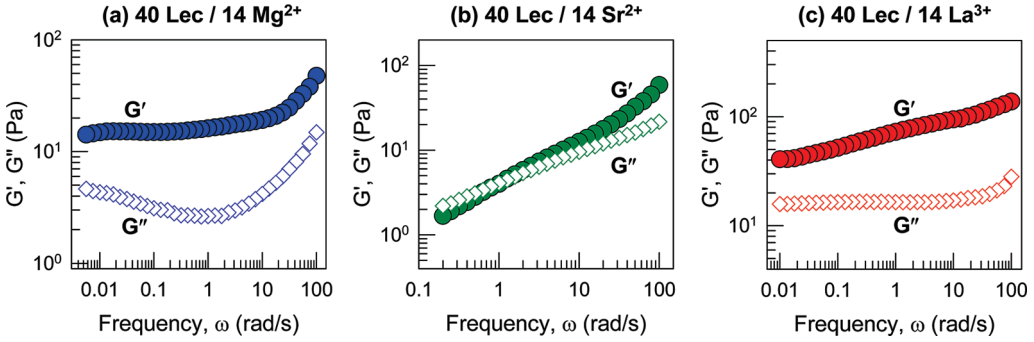

Figure 3. Rheology of lecithin samples containing various multivalent cations. Dynamic frequency sweeps are shown for samples in *n*-decane containing 40 mM of lecithin and 14 mM of (a) Mg^{2+}, (b) Sr^{2+}, and (c) La^{3+}. All samples are highly viscous or gel-like.

Table 1. Ability of Various Salts to Convert Lecithin Organosols into Organogels*

	compound	phase/state
cations : monovalent	NaCl	sols
(group 1, alkali metals)	KCl	sols
	CsCl	sols
cations : divalent	**$MgCl_2$**	**gels**
(group 2, alkaline earth metals)	**$CaCl_2$**	**gels**
	$SrCl_2$	**gels**
	$CaBr_2$	**gels**
	CaI_2	**gels**
cations : divalent	$CoCl_2$	sols
(transition metals)	$CuCl_2$	sols
	$CdCl_2$	sols
cations : trivalent	**$LaCl_3$**	**gels**
(lanthanides)	**$CeCl_3$**	**gels**
cations : trivalent	$FeCl_3$	sols
(others)	$AlCl_3$	sols

*All studies done in *n*-decane with 20−40 mM of lecithin and 0−40 mM of salt. Sol vs gel distinguished visually.

for inducing gelation. The next question is whether other salts can also serve as organogelators. We examined a number of other salts in this context, and selected rheological results are shown in Figure 3 for the same solvent (*n*-decane) and for concentrations of 40 mM lecithin and 14 mM of the respective salts. First, we evaluated other divalent cations along Group 2 of the periodic table. Both $MgCl_2$ and $SrCl_2$ were able to modulate fluid viscosity. The dynamic response of the Mg^{2+} sample (Figure 3a) is distinctly gel-like, with a flat (frequency-independent) G' and a nonmonotonic G''. Here also, the moduli do not intersect over the frequency range, indicating that the relaxation time is very high. In comparison, the Sr^{2+} sample (Figure 3b) shows a viscoelastic response, with G' and G'' intersecting around a frequency of 1 rad/s. In other words, Sr^{2+} appears to be a weaker gelator; to attain gel-like behavior, higher lecithin and Sr^{2+} concentrations are necessary. In addition to the Group 2 cations, we were also able to produce organogels using salts of the rare-earth lanthanides, specifically, La^{3+} and Ce^{3+}. Figure 3c shows the rheology of a lecithin/$LaCl_3$ sample, which again shows gel-like behavior with G' exceeding G'' over the frequency range.

Table 1 collects our observations with various salts contrasting those that induce organogelation and those that do not. The majority of these observations were made in the solvent *n*-decane, although similar trends hold for other solvents as well (see below). We found two broad patterns of behavior. In the case of gel-inducing salts, the progression with increasing salt concentration, as depicted in Figure 1, is from sol to gel, then back to near-sol (down the viscosity peak), and then the formation of a turbid, low-viscosity phase. This pattern was found for Mg^{2+}, Ca^{2+}, Sr^{2+}, La^{3+}, and Ce^{3+}. In the case of Ca^{2+}, in addition to $CaCl_2$, we also examined anhydrous $CaBr_2$ and CaI_2; gels were formed with these other anions as well. The second pattern of behavior was observed with the rest of the salts, which included salts of Na^+, K^+, Cs^+, Co^{2+}, Cu^{2+}, Cd^{2+}, Al^{3+}, and Fe^{3+}. All of these salts had no effect on the viscosity of lecithin organosols. When their concentration was raised too high, these salts could no longer be solubilized and excess (undissolved) salt precipitated out as solid crystals.

Organogels using the combination of lecithin and a multivalent cation like Ca^{2+} could be produced in a range of nonpolar organic solvents. In addition to *n*-decane, we have prepared gels in other *n*-alkanes, iso-alkanes, cyclohexane, fuels like kerosene, fatty acid esters, and aromatic solvents like styrene and divinyl benzene. The most efficient gelation (viscosity maximum) occurred at a lecithin:Ca^{2+} molar ratio ~3:1 in all the solvents we studied. All gels were indefinitely stable at room temperature. Gelation did not occur, however, in polar organic solvents like ethanol or other alkanols. In these polar solvents, the salts dissolved directly without the need for lecithin. Thus, for gelation to occur, the salt must be insoluble in the neat solvent and must dissolve only in the presence of lecithin. This ensures that the salt ions are sequestered in the polar regions of the reverse micellar structures formed by lecithin.

We have also characterized the temperature-dependent response of lecithin/Ca^{2+} organogels. As has been noted earlier, organogels tend to "melt"; that is, their rheology undergoes a sharp change (gel to sol transition) at a distinct temperature.[19,26] In contrast, wormlike micelles show a more gradual change in their rheological properties; the viscosity and relaxation time show a steady, exponential decrease over the range of temperatures.[12,26] In the case of lecithin/Ca^{2+} organogels, the temperature response is a hybrid of the above (data not shown). On heating, the gels do not "melt" at a distinct temperature; rather, their zero-shear viscosity gradually drops. However, the dynamic response has the same qualitative behavior as that shown in Figure 2a; that is, the relaxation time remains long and outside the window of frequencies probed. These aspects require more careful study and are not discussed further in this paper.

We should also point out that all the salts we studied were *anhydrous*, and so the results are not due to residual or bound water, either in the lecithin or in the salts. In our earlier study, we had determined using ^1H NMR that residual water is present in lecithin at a molar ratio of 0.9:1 (water:lecithin).[11,12] However, if we added lecithin alone to an organic solvent, the solution was nonviscous. Also, bound water cannot explain why we see organogel formation only with certain cations and not others.

Microstructure of the Gels from SANS. Next, we describe the results of SANS experiments on lecithin/Ca^{2+} organogels.

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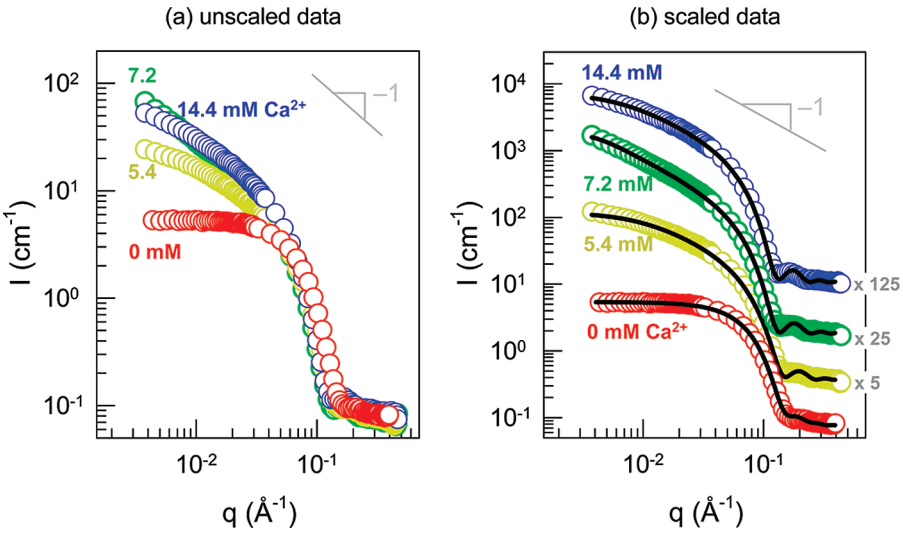

Figure 4. SANS spectra (intensity I vs wave-vector q) for samples in deuterated cyclohexane containing 20 mM lecithin and various concentrations of Ca^{2+}. In (a) the data are plotted on an absolute scale. Note that the intensity at low q increases from 0 to 5.4 to 7.2 mM Ca^{2+} and then decreases slightly for 14.4 mM Ca^{2+}. The same data are plotted in (b) with the different curves separated by factors of 5. Model fits (solid lines) are shown through each curve in this plot.

Table 2. SANS Model Parameters Obtained by Fitting Data in Figure 4

sample		ellipsoid param.		polydisperse cylinder param.		
Lec/Ca^{2+} (mM)	model type	R_a (Å)	R_b (Å)	R_c (Å)	L_0 (Å)	p_d
20/0	ellipsoids (oblate)	21.5	33.4			
20/5.4	polydisp cylinders			25.8	131.2 ± 2.3	0.99
20/7.2	polydisp cylinders			28.1	776.6 ± 7.6	0.24
20/14.4	polydisp cylinders			28.9	245.9 ± 3.1	0.84

These were conducted using deuterated cyclohexane as the solvent and with the lecithin concentration fixed at 20 mM. $I(q)$ plots are shown in Figure 4a for four different [Ca^{2+}]; for clarity, the same plots are shown scaled by factors of 5 in Figure 4b. In Figure 4a, we observe that the intensity at low q increases substantially as [Ca^{2+}] is increased. For samples with no or very low (< 3 mM) Ca^{2+}, the intensity tends to a plateau at low q. In contrast, the sample with 7.2 mM Ca^{2+} shows a power-law behavior close to $I \sim q^{-1}$ (slope of −1 on the log−log plot) at low q, which is the scaling relationship expected for rod-like structures.[12,23] Finally, note the slight drop in intensity at the lowest q for the 14.4 mM Ca^{2+} sample compared to the 7.2 mM one. The same data also shows more of a deviation from the $I \sim q^{-1}$ scaling at low q.

We have fitted form-factor models (described in the previous section) to the above SANS data, and the fits are shown in Figure 4b as solid lines through the data. Parameters from the modeling are shown in Table 2. For the 20 mM lecithin/no Ca^{2+} sample, the data admits to an oblate ellipsoid model (eq 1) with nearly identical major and minor axes (33.4 and 21.5 Å, respectively), indicating that the micelles are close to spherical.[11] For the other plots, a model for polydisperse cylinders (eq 2−6) is used. From the fit parameters, we note a small but distinct increase in the cylinder radius R_c with increasing [Ca^{2+}]: from 25.8 Å for 5.4 mM Ca^{2+} to 28.9 Å for 14.4 mM Ca^{2+}. Also, the average cylinder length L_0 sharply increases initially (from 131 Å for 5.4 mM Ca^{2+} to 777 Å for 7.2 mM Ca^{2+}) but then drops to 246 Å for the 14.4 mM Ca^{2+} sample. In other words, the cylinder length seems to increase and then decrease with [Ca^{2+}], which qualitatively reflects the trend in the zero-shear viscosity vs [Ca^{2+}] (Figure 1).

It is useful to compare the above parameters with those for lecithin-bile salt reverse wormlike micelles from our earlier study.[11] There also, the $I(q)$ for the lecithin-only sample was modeled as ellipsoids, and the model parameters were nearly identical. As the micelles grew upon addition of bile salt from ellipsoids to cylinders and then to worms, the cylinder radius remained constant and identical to that of the minor axis of the ellipsoid (22 Å). The average length of the cylinders, on the other hand, increased monotonically with the bile salt concentration. Here, we find that the cylinder radius is not a constant as a function of [Ca^{2+}] while the length increases and then decreases. Still, there does appear to be a correlation between the Ca^{2+} induced growth of cylindrical structures and the increase in sample viscosity (organogelation).

Mechanism for Ion-Induced Gelation. We now discuss the differences between various cations in inducing gelation of lecithin organosols. Ion-specific effects have been known for a long time in organogelators. One of the earliest examples was napalm, the material used in flame-throwers in World War II and Vietnam, which was a mixture of gasoline and the aluminum complex of difatty acids.[27−29] A variety of other organic molecules have been reported to act as organogelators when complexed with metal ions such as Fe(III) or Cu(II).[30−32] In most of these studies, the complex was first created by reacting the organic molecule and the metal salt (in water or other solvent). The isolated product of such a reaction (i.e., the complex) was then combined with organic liquids under heat and stirring to create

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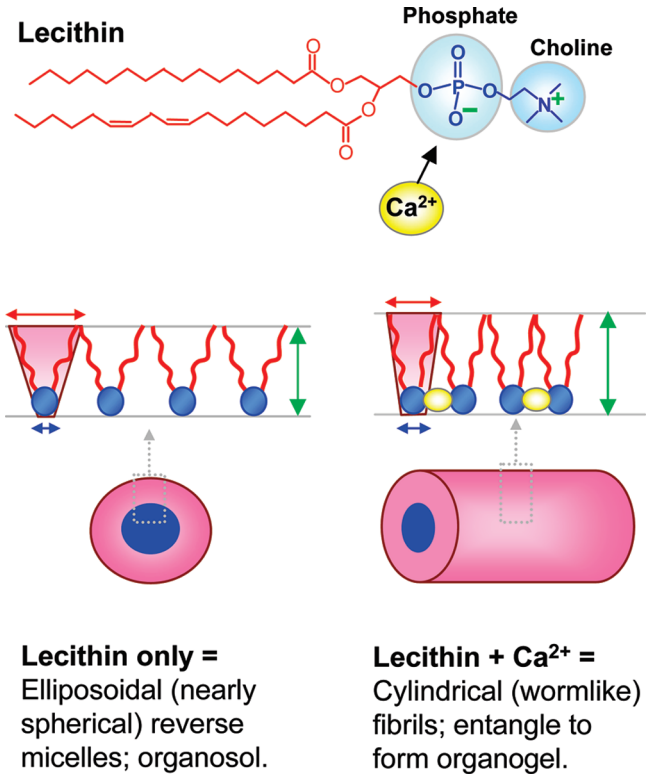

Figure 5. Effect of cations like Ca^{2+} on assemblies of lecithin in organic solvents. The top panel shows the structure of lecithin, highlighting its phosphocholine headgroup. Cations like Ca^{2+} bind to the phosphate portion of the headgroup. The bottom left panel reveals that lecithin alone has a conical shape and forms discrete, spherical reverse micelles. The bottom right panel depicts Ca^{2+} ions (yellow spheres) binding to lecithin (one ion per two lipids), causing the headgroup area a_{hg} (blue arrow) to expand. The lipid tails also become more straightened, causing the tail area a_{tail} (red arrow) to shrink and the micelle radius (green arrow) to increase. The molecular geometry becomes more like a truncated cone and in turn, the assemblies transform into cylindrical (wormlike) fibrils. The long fibrils entangle into a network, converting the sample into an organogel.

organogels. The metal ions for these complexes were chosen based on earlier binding/co-ordination studies, and thus only specific ions were investigated. For example, tetracarboxylate amphiphiles were complexed only with Cu(II)[30] while phosphonate esters were complexed with Fe(III) or aluminum.[31,32]

In the present study, we use a mixture of lecithin + salt as the organogelator; that is, we do not first isolate a complex of lecithin and a given cation. Also, we observe gel formation by a variety of cations, including alkaline earth metals (group 2 of the periodic table) and rare earth metals from the lanthanide series (La^{3+} and Ce^{3+}). The common feature of all these ions is their tendency to bind to the phosphocholine headgroups of lecithin. Over the years, numerous studies have examined the interactions between ions and phospholipids in water, both by experiments[13−16] and by simulations.[17,18] Their findings are highly pertinent here as well, even though our solvents are nonpolar organics. Akutsu and Seelig found that the binding affinity of cations to zwitterionic phospholipids followed the order $Na^+ < Ca^{2+} < La^{3+}$.[13] These ions were shown to associate with the polar headgroups of the lipids, and more specifically with the phosphate (negatively charged) portion of the headgroups, as illustrated in Figure 5.[13,17] Other studies have confirmed and extended the above order of cation-binding affinities. For example, Marra and Israelachvili showed that Ca^{2+} binds stronger to bilayers of phosphatidylcholines than Mg^{2+}, whereas the binding of monovalent cations like Na^+ was negligible.[15] Furthermore, in a study on micelles formed by C_8-lecithin in water, Huang et al. reported that Ca^{2+} had the strongest binding affinity among divalent cations.[16] Also, the affinity of La^{3+} was shown to be comparable to that of Ca^{2+} but lower than that of Ce^{3+}.[16]

Our results on organogel formation correlate with the binding affinities described above. Organogelation is induced by Ca^{2+}, but not by Na^+ or other monovalent cations. The elastic moduli G' of the gels are higher for Ca^{2+} than for Mg^{2+} or Sr^{2+}, indicating that Ca^{2+} is probably the most efficient gelator among the divalent ions (Figures 2, 3). Also, the G' values for La^{3+} are comparable to those for Ca^{2+}, indicating that La^{3+} is also a very efficient gelator. Taken together, our results reveal a systematic connection between phospholipid-cation binding and organogelation. Why should this be so? We know from SANS that cations like Ca^{2+} transform spherical micelles of lecithin into long cylindrical structures. This suggests a scenario where binding of Ca^{2+} alters the net geometry of the lipids to one favoring cylindrical structures at the expense of spherical ones. Similar arguments have been put forth in our earlier studies on lecithin/bile salt mixtures.[11,12]

The geometry argument can be framed 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 tail and headgroup, respectively.[1] Lecithin alone tends to have a p much greater than 1 in oil, implying an inverse cone shape, and this accordingly leads to near-spherical reverse micelles (Figure 5). A reduction in p due to either an expansion of the headgroup area a_{hg} or a shrinking of the tail area a_{tail} would transform the geometry into a truncated cone shape − and this would drive a transition from spheres to cylinders. It is possible for the binding of Ca^{2+} or other cations to expand the lipid headgroup area a_{hg}, especially since the multivalent nature of the cations implies simultaneous binding to more than one lipid at the same time (see below).[13,14] A second effect that could be important is that cation binding forces the lipid molecules together, which makes the lipid tails more densely packed and ordered.[17,33,34] The tails are effectively forced into a more straightened configuration and in turn, the average cross-sectional area a_{tail} per lipid may decrease.[33,34] Thus, cation binding may simultaneously affect both a_{tail} and a_{hg} in ways that cause a decrease in p, and this is depicted in Figure 5.

We should point out here that the cylindrical micelles or *filaments* in lecithin/ion mixtures appear to be very different from the reverse wormlike micelles seen, for example, in lecithin/water[9,10] or similar[11,12,35−37] systems. A characteristic property of wormlike micelles (both normal and reverse) is that they are "living" polymers: they rapidly break and reform due to exchange of amphiphiles between neighboring chains.[3,9] In the limit where the time scale for breaking τ_{br} is much shorter than that for chain reptation τ_{rep}, the micellar sample behaves as a viscoelastic Maxwell fluid with a single relaxation time.[3,9] Lecithin/ion samples, on the other hand, are gel-like (elastic), not viscoelastic, and their rheology is quite different from that of a Maxwell fluid. The most likely explanation is that the cylindrical lecithin/ion chains have very different dynamics. The binding of cations like Ca^{2+} to

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phospholipids is expected to be quite strong and this will hinder the facile exchange of lipids between the chains.[38,39] Therefore chain breaking and reforming will be significantly retarded (i.e., τ_{br} will be very large) − in effect, the chains will behave more like "dead" (static) rather than "living" (dynamic) polymers. The entanglement of these long, robust chains can then lead to a quasi-permanent gel network. The chains could still relax by reptation, but if they are long enough such relaxation will be very slow (the reptation time $\tau_{rep} \sim L^3$ where L is the contour length[3,12]). This could explain why a distinct relaxation time was not detected within the frequency window of our dynamic rheological experiments.

We also noted an increase in the radius of the cylinders with increasing [Ca^{2+}] from the SANS modeling. This was in contrast to the results for lecithin/bile salt mixtures where the micellar radius was independent of the bile salt concentration. The growing cylinder radius is likely due to the straightening of lipid tails mentioned above. Note that lecithin has two *cis* unsaturations in one tail, which implies kinks in that tail. When cations bind to lecithin, the lipid molecules are brought closer and this is believed to make the tails straighter and more ordered.[17,33,34] In the case of lipid bilayers in water, tail ordering has been shown to increase the bilayer thickness.[34] The same effect can increase the effective thickness of cylindrical chains in our lecithin/ion mixtures, and this is also shown schematically in Figure 5.

Lastly, past studies on lipid-ion binding can also help to explain the maximum in viscosity vs [Ca^{2+}] seen in Figure 1. Both experiments and simulations have shown that the binding between Ca^{2+} and zwitterionic phospholipids is optimized at a molar ratio of approximately 2 lipids per Ca^{2+} ion.[14,17] The viscosity maximum likely reflects this binding stoichiometry. As seen from Figure 1, the viscosity increases to a peak at a molar ratio of ~2.8 lipids per Ca^{2+} ion, which is somewhat close to the optimal binding ratio mentioned above. At this point, every Ca^{2+} ion is expected to be bound strongly to lipid headgroups, and this seems to correlate with the longest cylindrical micelles/filaments. Beyond this point, the presence of excess [Ca^{2+}] seems to cause a decrease in the cylinder length (as inferred from SANS modeling, Table 2), which correlates with the decrease in viscosity. The precise reason for the cylinder shortening is not clear, but it is possible that the excess Ca^{2+} stabilizes the end-caps of cylindrical chains (more end-caps means shorter chains). Alternately, excess Ca^{2+}, if still weakly bound to the lipid headgroups, may drive a transition from cylinders to reverse bilayers.

Lipid−ion mixtures in oil are thus analogous in many ways to the same mixtures in water. Oil-based systems may provide an ideal platform to study the interactions of biologically relevant lipids with various ions. From the viewpoint of organogelation, the potency of lipid−ion gelators could be further improved by rational choice of the lipid. For example, anionic lipids like phosphatidylglycerols (PG) or phosphatidylserines (PS) are known to interact much more strongly with cations like Ca^{2+} compared to the zwitterionic phosphatidylcholines.[33,38] Also, lipid-ion interactions are considerably stronger when the lipids are in their gel (frozen) state rather than in their fluid state.[15] Thus, we predict that an anionic lipid with long saturated tails, when combined with Ca^{2+}, could act as a very potent organogelator. Of course, the lecithin/Ca^{2+} system is probably by far the most suitable for applications, as discussed below.

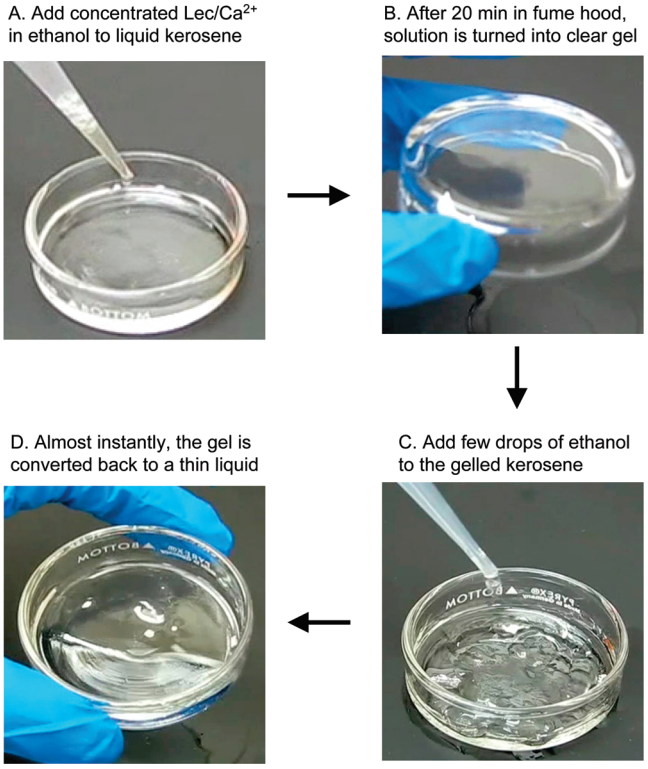

Figure 6. Isothermal gelling and ungelling of kerosene. The photographs are stills from movie 1 (Supporting Information). In panel A, kerosene is poured into a Petri dish, and to this is added a concentrated solution of the gelator (lecithin + $CaCl_2$ in ethanol in a molar ratio 20:7). The Petri dish is then placed in a fume hood for 20 min, whereupon the volatile solvent, ethanol, evaporates, and in turn, the liquid kerosene is converted into a clear organogel (panel B). The Petri dish is inverted to show the gel-like nature of the sample. To ungel (liquefy) this sample, in panel C, a few drops of ethanol are added. Almost instantly, the gel is converted back to a thin liquid, as can be confirmed from the tilted Petri dish (panel D).

Room-Temperature Gelling and Ungelling. Finally, we wish to briefly discuss the potential utility of using lecithin/ion mixtures as organogelators. It is worth reiterating how simple and inexpensive this combination is. Lecithin is a food-grade phospholipid that is frequently used in commercial and food products; it can be easily purchased in kilogram quantities. Lecithin/Ca^{2+} mixtures are biocompatible and nontoxic; they should thus be safe and gentle on the environment. The combination of low cost and biocompatibility could make these mixtures attractive for applications. One potential application is to convert liquid fuels into gels so as to store and transport these flammable liquids in a safe manner while avoiding problems of fuel leakage or spillage. However, a problem in many such applications is that the introduction of gelator into the solvent requires heat and/or shear. The gelator is usually mixed as a powder with the solvent and the mixture is heated to dissolve the gelator. To facilitate applications, it would be imperative to avoid this heating step.[31,32]

In this context, we describe how one could make gels using lecithin/Ca^{2+} without using either heat or shear. This process is depicted in Movie 1, which is included as part of the Supporting Information. We provide stills from this movie in Figure 6. The solvent we have used is kerosene, a typical liquid fuel. We took about 5 mL of kerosene in a Petri dish, as shown in Panel A. Separately, we prepared a concentrated solution of lecithin and $CaCl_2$ in ethanol (620 mM lecithin +220 mM Ca^{2+}), with the molar ratio roughly corresponding to the optimum for gelation.

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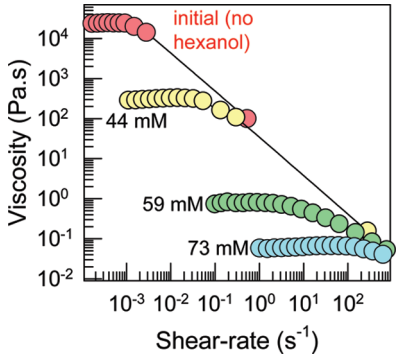

Figure 7. Ungelling of a lecithin/Ca^{2+} gel by addition of *n*-hexanol. Viscosity vs shear-rate plots are shown for a sample of 40 mM lecithin +14 mM Ca^{2+} in *n*-decane after addition of different amounts of *n*-hexanol (indicated beside each plot).

Because lecithin/Ca^{2+} does not gel ethanol, the ethanol solution was a thin liquid. Using a pipet, we added a small amount of the above solution to the kerosene such that the overall [lecithin] and [Ca^{2+}] in dry kerosene would be 40 mM and 14 mM respectively. The Petri dish was then placed in a fume hood, where the relatively volatile ethanol was allowed to evaporate. The evaporation was complete in about 20 min. In turn, the kerosene was converted into a clear gel, and this is shown in panel B. Neither heat nor any mixing or stirring were employed during the above gelation process. Also, instead of placing the sample in a fume hood, one could remove the ethanol in a few minutes by passing nitrogen gas over the sample.

An additional aspect is the ability to liquefy (ungel) the above kerosene gel as desired. For example, fuels may be stored in gelled form when not in use but may need to be liquefied for eventual use. Ungelling of lecithin/Ca^{2+} gels can also be done without heat or shear, and this is also depicted in movie 1 and Figure 6. Here, we make use of the fact that an alcohol like ethanol is a good solvent for calcium salts and can thus act as an ungelling agent. In panel C, we add a few drops of ethanol to the gelled kerosene. Instantly, we can observe the ethanol infiltrate the gel and begin to liquefy it. The Petri dish is gently shaken for a few seconds within which time the gel is completely converted into a sol (panel D). Thus, ungelling can be readily accomplished at ambient temperature. The above gelling-ungelling cycle can be repeated: if the ethanol added to ungel is removed in a fume hood, the sample will be gelled again.

The ungelling of lecithin/Ca^{2+} gels by alcohols can also be accomplished in a controlled manner (i.e., to intermediate extents). We have studied such ungelling using steady-shear rheology. For this purpose, it is convenient to use a less-volatile alcohol such as *n*-hexanol. Figure 7 shows data for a gel composed of 40 mM lecithin and 14 mM Ca^{2+} in *n*-decane, to which we add increasing amounts of *n*-hexanol. Addition of *n*-hexanol causes the zero-shear viscosity η_0 to be lowered systematically. With about 73 mM of *n*-hexanol, the η_0 is reduced by more than 5 orders of magnitude and the sample response becomes nearly Newtonian (i.e., a constant viscosity over the range of shear rates). Thus, a very small amount of alcohol is enough to liquefy the gel.

4. Conclusions

This study has demonstrated that simple inorganic salts can modulate the self-assembly of phospholipids like lecithin in organic solvents. Salt effects vary with the cation, and in particular, strong effects are found in the case of the divalent alkaline earth metals (Ca^{2+} and Mg^{2+}) and the trivalent lanthanides (La^{3+} and Ce^{3+}). These multivalent cations bind with the phosphocholine headgroups of lecithin and induce a transition from spherical reverse micelles to cylindrical fibrils. The fibrils have diameters ranging from ca. 4 to 6 nm while their lengths are expected to exceed several hundred nm. These fibrils behave like "dead", rather than "living" polymers; that is, they do not undergo frequent breaking and recombination events. The entanglement of these long, robust fibrils gives rise to a quasi-permanent gel network. Lecithin/ion gels can be formed in a variety of nonpolar organic liquids, and gel formation can be induced without heat or shear by use of a volatile cosolvent. These gels can also subsequently be ungelled by the addition of polar solvents like alcohols.

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Supporting Information Available: Movie depicting the isothermal gelation of kerosene. This corresponds to the images shown in Figure 6. This material is available free of charge via the Internet at http://pubs.acs.org.