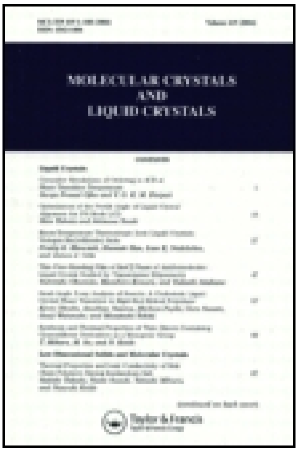

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Observation of a Nematic Phase in an Aqueous Suspension of Phospholipid Tubules

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We have found that at a sufficiently high concentration, diacetylenic phospholipid tubules form a nematic phase when suspended in water. As a consequence of their large size, the tubules were imaged with visible light and the nematic order parameter was extracted.

Keywords: nematic order, tubules, phospholipid

A number of years ago the diacetylenic lecithin 1,2-bis(10,12,tricosadiynoyl)-sn-glycero-3-phosphocholine ("$DC_{8,9}PC$") (Figure 1) was found to form microstructures in water consisting of bilayers which wrap around a hollow core.[1-4] These so-called "tubules" are straight, tens or even hundreds of microns in length, and 0.30–0.75 μm in diameter. The thickness of the walls ranges between one and fifteen bilayers,[5] and are generally uniform over an entire tubule or at least over large regions of the tubule. The diacetylene in the lipid tail can be easily polymerized, allowing the tubules to be extracted from the water without significant change in their macrostructure. Given their robustness both in and out of suspension, one can imagine a variety of applications ranging from their use in composites to microscopic stirring bars. On the other hand, from a purely scientific standpoint, the internal structure of the tubules and their interactions have been important areas of ongoing research.[6-10] Following our initial program of learning how to manipulate the tubules with external fields,[5,11-13] we have recently been studying interactions of tubule suspensions with bounding surfaces[14] and tubule-tubule interactions *in vitro*.[15] In the former work we calculated the separation-dependent partition function of the tubule-surface system, using acrylic as an example, assuming van der Waals attractions and short range hydration repulsive forces. Owing to the trade-off between energetic and entropic terms which arise from geometric effects, we showed that a hump of order a few k_BT exists in the Helmholtz free energy near the surface. Here k_B is Boltzmann's constant and T is the temperature. In effect the hump serves as a weak barrier against tubule binding, stabilizing the

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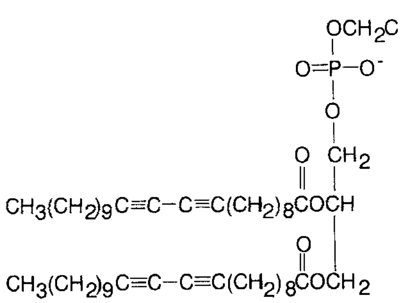

FIGURE 1 Schematic representation of $DC_{8,9}PC$ molecule.

suspension for relatively long times compared to a typical translational diffusion time. More recently we have investigated tubule-tubule aggregation into macroscopic structures best described as "log jams." By varying pH, we showed that in the mid pH range (pH > 3.5) the tubules form dilute aggregates over the course of several hours. Between pH ~ 3.5 and pH ~ 2.5 the tubules do not aggregate, and below pH ~ 2.5 they again aggregate. The data strongly suggest that van der Waals attractions are responsible for aggregation in the mid pH range. As the pH is reduced, however, the zwitterionic tubules gain a net positive charge. In consequence, in the pH region $2.5 < \text{pH} < 3.5$ the magnitude of the long range Coulombic barrier becomes sufficiently large relative to k_BT to prevent close approach and aggregation. Finally, at very low pH, we find that although the net tubule charge is large, the screening length κ^{-1} associated with the ionic strength of the acid is sufficiently small so as to inhibit Coulombic repulsions. Only at separations smaller than κ^{-1} are Coulombic interactions effective; at and below these short distances, however, the van der Waals attractions are dominant, and aggregation occurs.

Armed with this information about tubule interactions, we have investigated the possibility of forming a bulk nematic phase composed of these structures. At or near pH $= 7$ the tubules would tend to form aggregates, and it would not be possible to achieve a stable nematic phase. On the other hand, by judicious choice of pH we are able to stabilize the tubules. Since the effective range of the Coulombic interaction (as determined by κ^{-1}) is short relative to the actual tubule diameter, for all intents and purposes we can consider the tubules to interact via steric repulsions, with an effective diameter only slightly larger (by a factor of order $2\kappa^{-1}$) than the bare diameter. Nematic phases mediated by steric interactions of anisometric particles are, of course, well known. Several decades ago Onsager predicted an athermal isotropic-nematic phase transition based upon particle aspect ratio and concentration.[16] Since his calculation is valid only in the dilute, large aspect ratio regime, there has been tremendous activity over the years to improve upon Onsager's quantitative predictions in the limit of dense, stubby particles.[17-24] Even so, Onsager's qualitative results have been experimentally realized in systems such as aqueous suspensions of tobacco mosaic virus (TMV) and poly benzyl glutamate.[25-27] The viscoelastic properties of these colloidal nematic phases have been investigated by light scattering,[28,29] and more recently Zasadzinski, *et* *al.* created beautiful freeze-fracture electron micrographs of the nematic phase of TMV.[30,31] These investigations have uncovered a number of new and exciting physical phenomena associated with large aspect ratio rigid particles, and improved our understanding of concepts as fundamental as elasticity. Despite these significant accomplishments, however, the relatively small size of polyelectrolytes such as TMV still precludes the use of visible light for purposes of imaging, and prevents the manipulation of the nematic one particle at a time. From this perspective the tubules offer a possible alternative, since they are easy to manipulate and under many circumstances, can be imaged with visible light. The purpose of this paper is to report on the observation of a nematic phase of tubules and discuss some of its ramifications.

The phospholipid $DC_{8,9}PC$ was obtained from Avanti Polar Lipids and used without further purification. 10 mg of lipid was added to 10 ml of solution consisting of absolute ethanol and water in a ratio 70:30 by volume. The mixture was heated to ~60°C, then removed from the oven and allowed to cool. After approximately 1 h the mixture had become extremely cloudy. Examination of the material using optical microscopy revealed the presence of needle-like structures tens of microns long, the signature of tubules. The suspension was then centrifuged and the water-ethanol supernatant was withdrawn. Pure water was then added, the tubules re-dispersed, the suspension centrifuged, and the supernatant again withdrawn. This process was repeated four times in order to remove the ethanol from solution. The final step involved resuspension of the tubules to a concentration of 10 mg/ml in a mixture of H_2O, D_2O, and hydrochloric acid. The density of the suspending medium was 1.093, equivalent to the mass density of the tubules,[15] and the pH was 2.75, which is within the non aggregation regime.[15] At this pH, moreover, we would expect the effective repulsive diameter of the tubule to be only slightly larger than the excluded volume diameter since κ^{-1} is of the order of 100 Å.

A small quantity of tubules in suspension was placed between two microscope slides. By means of optical microscopy we determined the length distribution of tubules, which is shown in Figure 2; from 214 individual tubules we found the average length L to be 29 ± 7 μm. Assuming an average diameter $d \sim 0.5$ μm and an average wall thickness of ~125 Å[5] (corresponding to approximately $2\frac{1}{2}$ bilayers), we find the tubule number density $\rho \sim 1.6 \times 10^{10}\ \text{cm}^{-3}$ in suspension. In determining ρ we used the experimentally determined tubule mass density of 1.093 g/cm^3 and a sample concentration of 10 mg/ml, as established above. This concentration, moreover, is quite high, especially when compared to the maximum number density $\rho_{\max} \sim 1.58 \times 10^{11}\ \text{cm}^{-3}$ obtained if the tubules were arranged in a close packed structure. It is in this concentration region, then, that we might expect to see a nematic phase.

A quantity of suspension was injected by capillary action between a pair of acrylic slides separated by 175 μm mylar spacers. Through experience[14] we have found that the tubules tend to bind rapidly to glass (most likely due to a surface charge on the glass), but much more slowly to acrylic. The sample was then placed in the bore of a superconducting magnet at field $H = 7.8T$ for 10 h, with the direction of the field in the plane of the sample and parallel to the original flow direction. Note that the entire experiment was performed at room temperature, approxi-

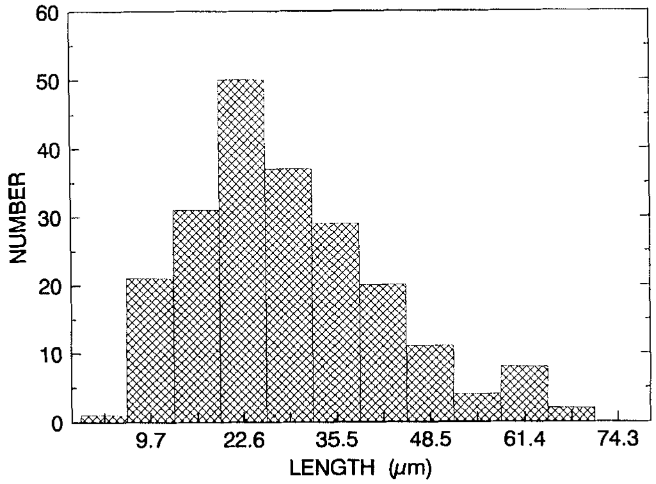

FIGURE 2 Length distribution of tubules.

mately 22°C. The sample was removed and observed under an optical microscope. Despite the entropic barrier which *retards* (rather than prevents) tubule binding to a surface, we observed that some tubules at the two acrylic surfaces tended not to align with the field, but rather adopt a random orientation. We surmise that these tubules early on had irreversibly become bound to the walls, and that the magnetic field had little or no effect on their orientation. Nevertheless, in general we found that the number density near the walls tended to be comparable to that in the center region, and thus it is unlikely that tubules were depleted from the center of the sample. As the microscope was focused in the central part of the sample, we observed large regions of well aligned individual tubules, with a nematic correlation length having a *lower limit* of ~250 μm. The quality of the image was, of course, determined partially by the depth into the sample of our focus, the optical depth of field relative to typical tubule separations, and the overall thickness of the sample. Bearing in mind that the tubules have a large scattering cross section, it's difficult to form a perfectly clear image of the tubules unless one were imaging only a single layer. In such a scheme, of course, two complications arise. First, a thin layer between rigid walls would quickly find the tubules irreversibly bound to the surfaces.[14] Even if this were avoidable, a thin layer would no longer represent a three dimensional system. These quasi-two dimensional systems are themselves intrinsically interesting, and in consequence we are currently making Langmuir films on the surface of pure D_2O; this work will be reported elsewhere.

Figure 3 shows a photograph of the sample taken approximately 750 h after magnetic alignment and focused at a depth approximately one-half the sample thickness. The unfocused dark regions correspond to locally slightly higher concentrations of tubules, especially near the two surfaces. Owing to the limited depth of field at even moderate magnification and the inherent graininess of the photograph, it's much easier to discern dark streaks corresponding to the local tubule orientation rather than individual tubules in the photograph. Nevertheless, the imagable region of the sample appears to be well-aligned, most likely in the nematic

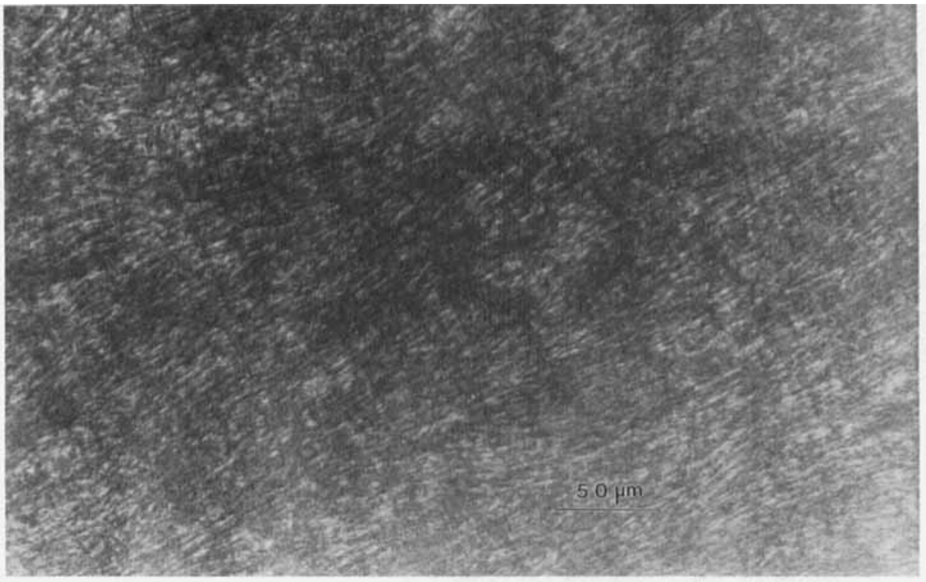

FIGURE 3 Photograph of a region of aligned tubules. The bar represents 50 μm.

phase. A weak bend distortion seems to be present. In other regions of the sample we believe we have observed splay, although with insufficient confidence to warrant inclusion. For pedagogic purposes, we show in Figure 4 a photograph of a sample which is at pH ~ 7 and is approximately 7500 h old. Over the course of time the well-aligned tubules in the nematic phase had translationally diffused and, because of the pH, become bound to both the walls and to each other. The focus of the photograph is at one surface. Because of the large number of tubules at this surface and because those at the other surface are *considerably* out of focus, a much clearer image was created. The nematic order of the vast majority of tubules is readily apparent; those few tubules aligned in other directions had early on become bound to the walls, as discussed above.

We have attempted to extract an orientational order parameter for the nematic phase. The in-plane projection α of the polar angle θ between the local director and the tubule axis was measured at 356 points for the sample shown in Figure 3; the results are shown in Figure 5. From the data, an in-plane order parameter $\langle P_2(\cos\alpha)\rangle \sim 0.96$ was measured. If the alignment is uniaxial, one can easily show that $\langle \tan^2\alpha\rangle = \frac{1}{2}\langle \tan^2\theta\rangle$. Moreover, if we assume that $\langle \tan^2\theta\rangle + 1 = \langle 1/\cos^2\theta\rangle$, we find that the two-dimensional order parameter corresponds to a three dimensional value $\langle P_2(\cos\theta)\rangle \sim 0.92$. These values are quite large, although they are quantitatively consistent with those reported by Zasadzinski, *et al.* for TMV[30,31] using freeze-fracture and by Oldenbourg, *et al.*[26] for TMV using x-rays.

In the limit of very long and dilute particles, Onsager has calculated the condition on density for the existence of a nematic phase.[16] Normalizing his result to the close packed density, he found that the required packing fraction for a nematic phase is $\rho^* = 4.9\, d/L$, where ρ^* is defined as ρ/ρ_{max}. More recently Lee calculated[17]

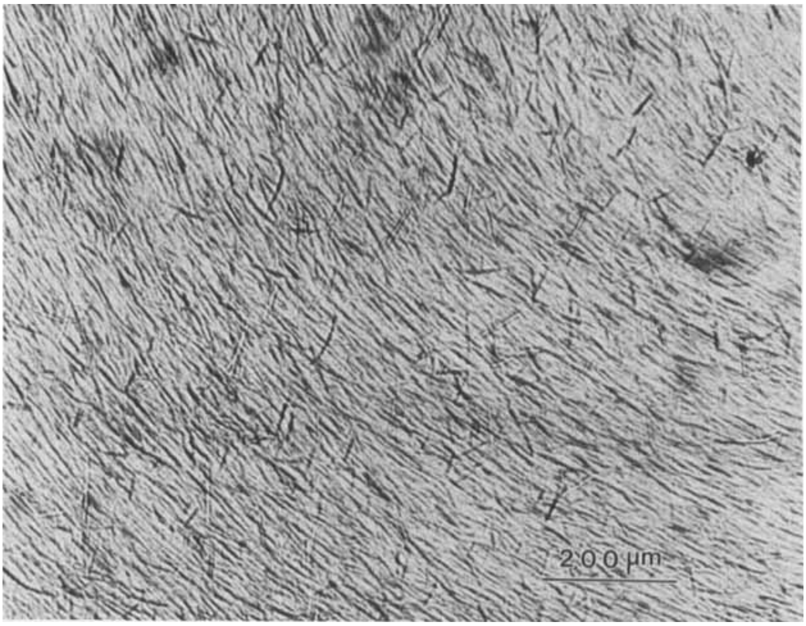

FIGURE 4 Photograph of a 7500 h old sample in which the nematic tubules ($L \sim 50$ μm) had become bound to the walls. The bar represents 200 μm. See text for details.

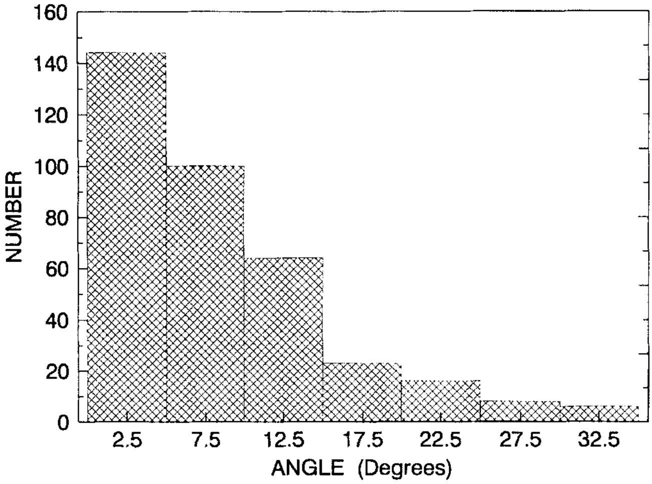

FIGURE 5 Distribution of orientations of individual tubules about the local average director.

a generalization of the Carnahan-Starling equation of state for the free energy of a fluid of hard spheres, reproducing Onsager's results in the appropriate limit. Lee's results, moreover, are consistent with molecular dynamics calculations of hard ellipsoids at significantly higher concentrations.[32] Given the very large average aspect ratio of our tubules ($L/d = 58$), it turns out that both the Onsager and Lee predictions are in good agreement, predicting a minimum nematic concentration $\rho^* \sim 0.084$. Since the reduced concentration ρ^* used in our experiment was 0.101, it appears that, based upon theory, the sample is indeed in the nematic phase. Caution, of course, must be used. If the average wall thickness were larger, then the tubules would contain more lipid and the overall *number* density of tubules would be reduced. Although there is a paucity of data on tubule thicknesses, our numbers are consistent with previous electron micrography results. Note, however, that thicker-walled tubules have been reported.[33] In addition, if the average diameter were larger than 0.5 μm, the condition for ρ^* would increase proportionally. Both slightly larger and smaller diameter tubules have been observed and reported in the literature,[4,5,33] with a value $d = 0.5$ μm being quite typical. Finally, to our knowledge no theory extant considers length distributions in determining critical concentrations or order parameters. It may be possible, for example, that longer tubules exert proportionally more influence than their shorter counterparts on the formation of a nematic. The value reported above for the average length (29 μm) represents a number average, viz, $L_n = \Sigma L_i/N$, where L_i is the length of tubule i. On the other hand, if a length averaged value of L were more appropriate, i.e., $L_l = \Sigma L_i^2/N\Sigma L_i$, we would find an average length $L_l \sim 35$ μm, perhaps resulting in a lower scaled concentration necessary for the formation of a nematic phase. Thus, with these caveats in mind, it does appear that we have observed a nematic phase of tubules, a result which seems to be consistent with theoretical predictions based upon our concentration data.

As an additional check, we note that if the sample were not nematic but instead were out of equilibrium and only temporarily aligned, over the course of time rotational diffusion would result in a sample which appeared isotropic. Doi and Edwards have derived[34] an expression for rotational diffusion of densely packed rods, finding a rotational diffusion constant

$$D_r = \beta D_{ro}(\rho L^3)^{-2}. \tag{1}$$

Here D_{ro} is the usual rotational diffusion coefficient in the limit of low density,

$$D_{ro} = \frac{3k_BT}{\pi\eta L^3}(\log_e L/d - \gamma)$$

where η is the viscosity of the suspending medium and γ is a parameter which accounts for end effects. Although many expressions for γ have been given, we take the empirical form of Tirado and de la Torre,[35] $\gamma = 0.662-0.92\,(d/L)$, which has been shown to produce good results over a wide range of aspect ratios. Finally, Doi and Edward's parameter β is empirical, and for a wide class of materials and aspect ratios, has been found to be approximately 1000.[36] It should be noted that this expression for D_r is valid in the concentration regime $L^{-3} < \rho < (dL^2)^{-1}$. The first condition implies that there is a significant number of tubule overlaps, whereas the second is a condition which states that the concentration is below that corresponding to the nematic-isotropic phase transition. The first condition is easily met, whereas the second condition is, by experimental design, not met. *If* the sample were in the isotropic phase and the concentration conditions were satisfied, we would find from Equation 1 that the characteristic rotational diffusion time $\tau = (6D_r)^{-1} \sim 6$ h. Since the second condition is not satisfied, however, τ is undoubtedly larger, although we know of no model which describes this regime. Nevertheless, over a time scale of order 750 h we found that the bulk of the tubules remained well aligned in the nematic phase. Small regions of more random alignment were observed, perhaps arising from pinned surface strains, locally reduced concentrations due to some tubules translationally diffusing and becoming pinned at the surfaces, or perhaps biphasic equilibrium. The important point, however, is the long term stability of the alignment.

As a final note, we pointed out earlier that the nematic correlation length ξ has a lower limit of approximately 250 μm, corresponding to about eight tubule lengths. This ratio is comparable to typical low molecular weight thermotropic liquid crystals in the isotropic phase just above the nematic transition. Given that the discontinuity in, for example, the nematic order parameter is much larger for the tubules at the NI transition than it is for thermotropics, we suspect that *if the tubules were isotropic*, ξ would be considerably smaller than 250 μm. The apparent finite size of ξ most likely reflects the pinned defects described above.

In conclusion, we have observed what appears to be a bulk nematic phase and measured the order parameter in a dense suspension of large phospholipid tubules. Such a phase is predicted by a number of athermal theories for elongated rods. The large size of the tubules makes this an exciting system for the study of a number of properties of nematics, which is the subject of our current work.

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