Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

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Paul Yager [a] & Paul E. Schoen [a]
[a] Code 6510 Optical Probes Branch Naval Research Laboratory Washington, DC, 20375, U.S.A.
Version of record first published: 20 Apr 2011.

To cite this article: Paul Yager & Paul E. Schoen (1984): Formation of Tubules by a Polymerizable Surfactant, Molecular Crystals and Liquid Crystals, 106:3-4, 371-381

To link to this article: http://dx.doi.org/10.1080/00268948408071454

Mol. Cryst. Liq. Cryst., 1984, Vol. 106, pp. 371-381
0026-8941/84/1064-0371/$18.50/0

Printed in the United States of America

FORMATION OF TUBULES BY A POLYMERIZABLE SURFACTANT

PAUL YAGER AND PAUL E. SCHOEN

Code 6510
Optical Probes Branch
Naval Research Laboratory
Washington, DC 20375
U.S.A.

ABSTRACT

The phospholipid 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine, in which both fatty acyl chains contain polymerizable diacetylenic units, has been studied with regard to its behavior in aqueous dispersion before and after polymerization. The monomeric lipid may be dispersed in distilled water above its chain melting transition temperature, but, contrary to previous reports, it does not stay in liposomal form on subsequent reduction of the temperature. Microscopic observation shows formation of structures resembling so-called cochleate cylinders, except that these cylinders are water-filled. These "tubules" reversibly convert to liposomal form on heating above the monomer chain melting temperature. However, on polymerization with ultraviolet light, the cylinders are "locked in" and no morphological changes are observed on heating. These unique structures may represent a new class of orientable polymers.

INTRODUCTION

Phospholipids self-organize into a variety of structures with dimensions on the micron and submicron scale. These structures, by themselves or in combination with proteins, are obvious candidates for the building blocks in the developing field of biomolecular engineering. They are quite sensitive to temperature, pressure and surface interactions. While this characteristic sensitivity affords the opportunity to tailor the geometry and size of the structures formed, it also severely limits the tolerable environmental conditions. The introduction of polymerizable phospholipids provides a way around this limitation. Structures of interest can be formed from the monomers and then ruggedized by polymerization. Polymerizable surfactants are being studied in many areas of science, as they have potential uses in such diverse areas as drug delivery with stabilized liposomes[1], Langmuir-Blodgett multilayer technology for lithography[2,3], photographic applications[4] and one and two dimensional electrical conduction[5].

We present here partial results of the first phase of a broad-based research program on polymerizable surfactants--an investigation of properties of a phospholipid with two diacetylenic fatty acyl chains. The topotactic polymerization of diacetylenes requires accurate alignment of the monomers, so that such reactions do not proceed in the melt[6]. Recent studies on phospholipids in which one or more of the hydrocarbon chains contain diacetylenic units have shown that the production of red polymer occurs below the hydrocarbon chain melting transitions of such systems[1,4,7-9]. It has been assumed that the low temperature phase of such lipids is identical to that seen in phospholipids with fully saturated hydrocarbon chains with comparable melting transition temperatures. This would result in the production of polymerized liposomes with one or many bilayers, depending on the treatment of the monomer dispersion prior to polymerization. We have applied the techniques of optical absorbtion spectroscopy, Raman and infrared spectroscopy, calorimetry, optical microscopy and freeze fracture electron microscopy to the study of aqueous dispersions of a polymerizable lipid before and

after polymerization. The monomer lipid in aqueous dispersion has a reversible phase transition. Above the transition temperature the lipid is organized into the usual liposomal structure. Below the transition temperature a novel structure can form. It is a hollow cylinder of about one micrometer diameter and several tens of micrometers long. "Tubules" can be stabilized by polymerization, and spectral evidence suggests that the polymerization reaction proceeds in a manner similar to that in simpler diacetylenic monomer materials.

MATERIALS AND METHODS

The fatty acid 10,12 tricosadiynoic acid was synthesized by J P Laboratories, and the iosomerically pure phospholipid (1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine ($DC_{23}PC$)) derived from this fatty acid was esterified and purified by Avanti Polar Lipids. The resultant phosphatidylcholine gave a single spot by thin layer chromatography. The chloroform solution of the lipid was dried in vacuum, then dispersed in filtered deionized water by agitation at temperatures above 50° C. Calorimetric studies were performed on a Perkin-Elmer DSC-2, and spectrophotometry on a Cary 219C. Lipids were polymerized by exposure to a Spectroline mercury vapor lamp. Optical microscopy was performed with a Leitz Ortholux I microscope outfitted with long working distance objectives for phase and interference contrast. Temperature of the samples for optical microscopy was controlled by a Bailey TS-2ER heating and cooling stage. Samples for electron microscopy were frozen from room temperature by rapid immersion in Freon 22 at its melting point. Fracturing and replication was done at -105° C in a Balzers 301 Freeze Fracture device, and replicas were photographed in a Philips 200 transmission electron microscope.

RESULTS AND DISCUSSION

Calorimetry of unpolymerized lipid in pure water gave a single endotherm on heating from 0° to 80°C (see Figure 1). The peak of the endotherm occurred at 42°C, ±0.2, but its intensity was sensitive to the history of the sample, being smaller when rescanned immediately. Prolonged cooling below 0°C enhanced the endotherm, and prolonged heating above 70°C reduced the endotherm, and

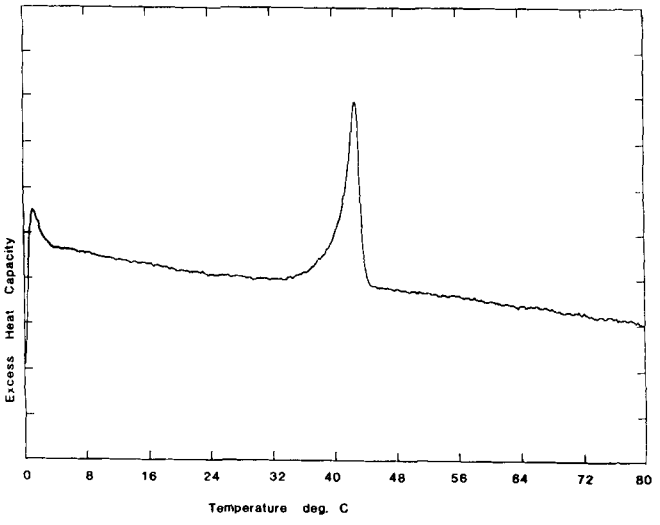

Figure 1. Differential scanning calorimetric record from a dispersion of unpolymerized $DC_{23}PC$. The peak of the single endotherm is at 42°±0.2°C. The calorimeter was scanned at 2.5°/minute at a gain of 0.5 mcal/sec.

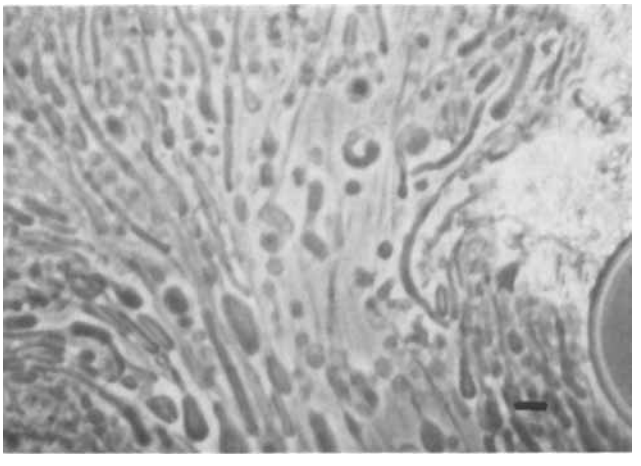

Figure 2. Phase contrast optical micrograph of an unpolymerized $DC_{23}PC$ dispersion above 42°C. The appearance is typical of liposomes of fluid phospholipids. The bar represents 10μm.

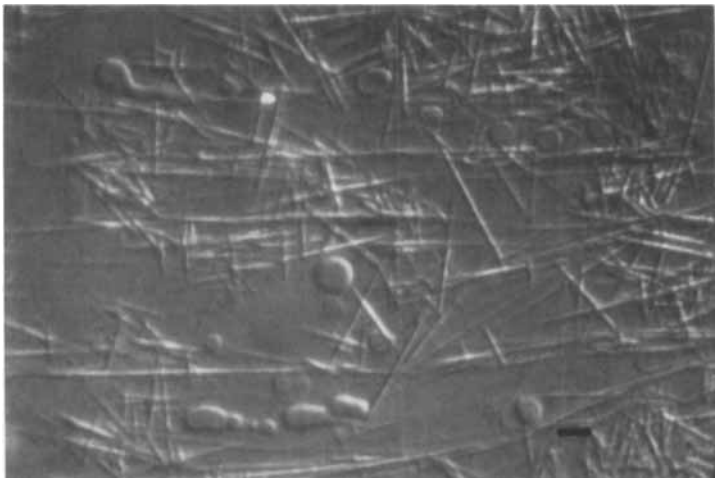

Figure 3. Interference contrast optical micrograph of a dispersion the temperature of which has just been lowered to about 39°C. Liposomes are seen to convert to tubule-shaped structures. The bar represents 10μm.

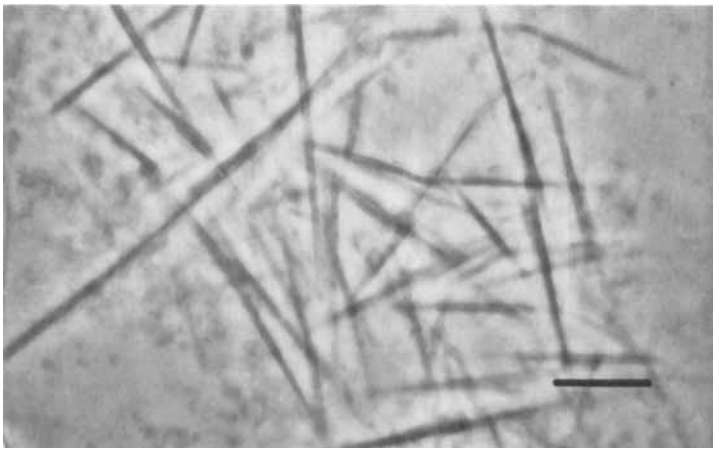

Figure 4. Phase contrast optical micrograph of unpolymerized tubules at high magnification. Note that tubules appear hollow. The bar represents 10μm.

differences in peak area of as much as a factor of four were observed between subsequent scans on the same sample. The asymmetric shape of the endotherm is not typical of that seen for phospholipid dispersions, and our transition temperature is higher than that reported by Johnston et al.[7] We have not resolved the reasons for this discrepancy. Polymerization of such samples by irradiation at room temperature resulted in some reduction of the peak intensity, but the continued presence of an endotherm at 42°C indicated that the degree of conversion must be low despite intense coloring of the dispersion. The optical absorption spectrum of polymerized dispersions of $DC_{23}PC$ was similar to that of the "red" form of polydiacetylenes observed elsewhere[1] On heating to 60°C the doublet around 500 nm collapsed into a single, somewhat blue-shifted band, and on slow cooling the red-most band returned, somewhat enhanced over that seen originally, presumably because of rearrangement of the diacetylene backbone during annealing.

To check the observation of others that liposomes of diacetylenic phospholipids are not disrupted by the polymerization process[1,4,9], we monitored the endothermic transitions of the monomeric lipid under the optical microscope. We discovered that above the melting transition structures clearly recognizable as liposomes predominated (see Figure 2), but, to our surprise, on cooling the liposomes disappeared, sometimes violently, being replaced predominantly by tubular structures (see Figure 3). The formation of the tubules was enhanced by lowering the temperature slowly through the melting point, and holding the sample between 38° and 40°C for a few minutes. Rapid cooling to temperatures below 30°C inevitably resulted in violent conversion of liposomes to amorphous highly birefringent material similar to that seen when samples were stored frozen for some time. It was also observed that prolonged heating of samples at temperature greater than 70°C caused reduction of the size of the liposomes to the point that tubule formation was greatly reduced (replaced by formation of amorphous material mentioned above) no matter how long the temperature was kept near 39°C.

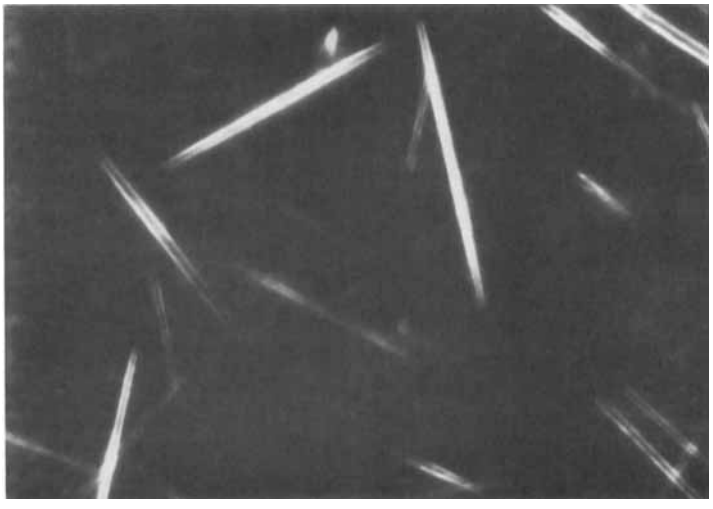

Figure 5. A sample similar to that seen in Figure 4, viewed in darkfield illumination. Magnification the same as that in Figure 4.

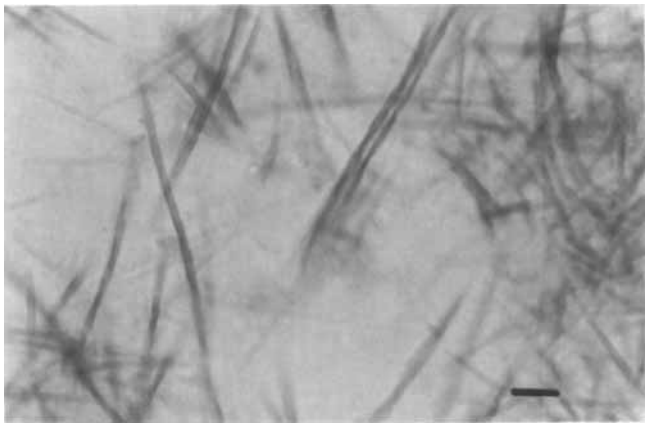

Figure 6. Brightfield optical micrograph of polymerized tubules. Note the slight twist induced in some tubules. The bar represents 10μm.

The conversion of liposomes to tubules was sufficiently slow as to be observable by eye under the microscope. It appeared that after nucleation a tubule would grow by rolling up pieces of bilayer which were torn from the outermost layers of multilamellar liposomes. At high magnification under the optical microscope more details of the structure of the tubules could be resolved (see Figures 4 and 5). The tubules were always a micrometer in diameter, and up to hundreds of micrometers long, and appeared to be quite straight and rigid. Furthermore, while certain properties varied from tubule to tubule, such as length and apparent wall thickness, the tubules always appeared open-ended. On heating, these structures readily converted to liposomes.

On exposure to the mercury vapor lamp, all non-fluid lipid samples, whether they were of the "amorphous" appearance or tubular, became red, indicating the formation of polymer. Polymerized tubular structures, particularly those which had been subject to shearing forces in suspension, were often not as straight as their monomeric precursors, and occasionally they had a semiregular twist along their lengths (see Figure 6). No change in structure could be seen in polymerized tubules between 0° and 60° and they could be air-dried or lyophilized without loss of structure. They were also highly birefringent, indicating ordering of the polymer relative to the tubular axis. Freeze fracture images of the monomeric tubules confirmed that they were indeed hollow cylinders containing water, surrounded by a cylindrical shell of a few layers of bilayer lipids; hence the choice of the name "tubules" (see Figure 7).

The deposition of unpolymerized tubules on a replica surface allowed observation of the structures by transmission, but because the electron bombardment caused rapid polymerization only polymerized tubules could be studied. As seen in Figure 8, the tubule is hollow and the polymerization induces a helical pattern of fibrous aggregation in the plane of the tubule wall. The surfaces of the monomeric tubules as seen in the previous Figure, have no such pattern. This texturing can be most easily explained by a change in the lipid packing caused by polymerization, distorting the packing of the hydrocarbon chains along the polymer axis. The alignment

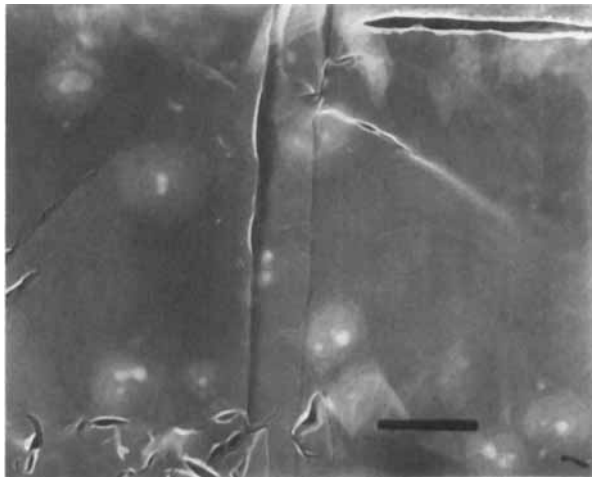

Figure 7. Transmission electron micrograph of a replica of an unpolymerized tubule. The fracture plane tends to follow the bilayer midplanes, and several fractured bilayers can be seen along the surface of this tubule. The bar represents 1μm.

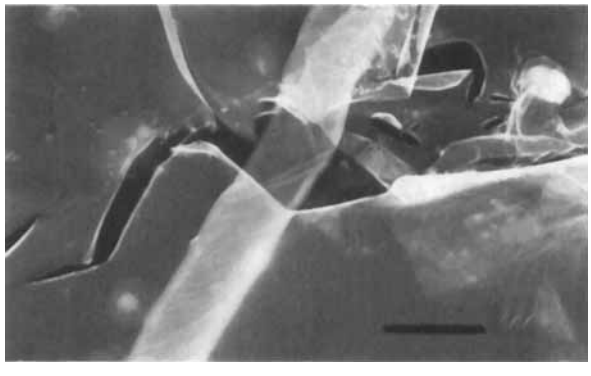

Figure 8. Transmission electron micrograph of a tubule lying across a tear in the replica. The tubule was unpolymerized when it was dried onto the film, but was presumably polymerized during the course of observation by the electron beam. Note the striations at 45° to the tubule axis. The bar represents 1μm.

of diacetylenic monomer units prior to polymerization will control the excitation of the polymer, and the spontaneous formation of the highly anisotropic tubules argues in favor of anisotropic packing of hydrocarbon chains within the shell of the tubule. There may be a corelation length over which adjacent diacetylenic units are in proper registry to allow polymerization, which could be quite long in helices circling the cylinder and quite short in other directions. The result would be a tendency for polymerization to propagate generally along the "long correlation length" helices. Although we have not yet determined the direction of the polymer chains relative to the tubule axis, it is probable that there is a regular arrangement of the polymer, and that the polymer backbone spirals around the tubule at an angle near 45° to the tubule axis.

These tubules differ from "cochleate cylinders" formed in phosphatidylserine dispersion in the presence of divalent cations in that the $DC_{23}PC$ tubules lack a tightly-coiled lipid core. Furthermore, they require no additional ions for their formation. Their formation is presumably driven by the steric requirements of the diacetylenic group, which puts a six-carbon linear segment in the middle of the chain. The formation of the tubules by rolling up bilayers from existing liposomes and the apparent minimum tubular diameter of a fraction of micrometer may explain why no such structures can be formed from liposomes smaller than a few micrometers in size. The formation of tubules is not "normal" for phospholipids, even those with extremely long hydrocarbon chains. We found that neither di-nervonoyl phosphatidylcholine (24:1, 15-cis) nor di-lignoceroyl phosphatidylcholine (24:0) form tubules under conditions similar to those used with $DC_{23}PC$.

The tubules are of interest not only because they represent another unusual behavior of lyotropic liquid crystals, but because the diacetylenic polymer may be oriented in a unique manner by this structure. Further studies of this system by spectroscopic and other methods are underway.

Acknowledgment: We would like to thank Dr. Russell. L. Steere and Dr. John E. Rash for instruction in freeze fracture technique. This work was partially supported by the Air Force Office of Scientific Research and the Naval Air Systems Command.

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