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Filamentous Bacterial Viruses VIII. Liquid Crystals of fd

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Abstract—Concentrated suspensions of filamentous bacterial viruses form cholesteric liquid crystals. Under appropriate conditions, spherulites are observed. The twisted nematic phase shows form optical rotation. In dry gels, where the pitch of the twisted layer is comparable to the wavelength of light, Bragg reflection from the sheets is observed.

1. Introduction

The fd-type filamentous bacterial viruses[9] are flexible deoxyribonucleoprotein rods, measuring about 60 by 9000 Å, and weighing about 19×10^6 amu. The 3000-odd major coat protein molecules in the virion (the virus particle), which account for about 85% of the weight, consist largely of α-helices, with the long axes of the α-helices oriented roughly parallel to the long axis of the virion. The single-stranded nucleic acid is encased in a tube of these α-helices, with the planes of the purine and pyrimidine rings of the nucleic acid at an angle of about 70° to the long axis of the virion. Since the nucleic acid molecule is circular, there are two such tubes running the length of the virion, joined at the ends.[8,9]

Concentrated amorphous solutions of the virion develop birefringent paracrystalline regions after standing for a few days.[7] Such concentrated gels can be dried into oriented crystalline fibers suitable for X-ray diffraction studies of molecular structure.[8] We report here some further observations on the liquid crystalline state of fd suspensions, which were made while investigating the conditions necessary to prepare improved specimens for X-ray diffraction. Some of these results have been briefly reported before.[9]

2. Materials and Methods

Stocks of fd were prepared and purified as described.[10] Concentration was determined by measuring the absorption of the suspension at 260 nm (OD_{260}), using appropriate dilutions, and employing the fact[7] that a 1 mg/ml solution with a path length of 1 cm has an OD_{260} of 3.74.

Quartz capillaries are produced by Paul Raebiger, Berlin-Spandau, and distributed by Unimex-Caine, Chicago.

Fibers of fd were prepared[8] by suspending a drop of concentrated gel (100 mg/ml or greater) between the tips of two glass rods, placed about 1 mm apart, and allowing the gel to dry slowly in a controlled-humidity atmosphere. The fiber was then placed in a quartz capillary, distilled water was added, and the ends of the capillary sealed with wax.

Gel specimens were aspirated into capillaries, and the ends either left open to allow the gel to dry[8] or sealed with wax.

In some cases, Bragg reflection was studied using gels placed in a thin chamber between two glass slides.[15]

Specimens were studied in aqueous suspension at ionic strengths below 0.01 M and at pH values ranging from 4.6 to 12.0. No gross effect of pH was noted, but the effect of this parameter was not studied systematically.

The dispersion of optical rotation, and the Bragg reflection from iridescent gels, were measured using filtered monochromatic light from a mercury lamp, except in the red, where a tungsten lamp with a Wratten 70 filter was used. Both a Zeiss polarizing microscope and a Wild binocular microscope with a polarizer and analyser attached were used. The apparent optical rotation θ' is the angle through which the analyser had to be rotated away from its original position, perpendicular to the polarizer, in order to give extinction of the polarized light incident on the specimen.

3. Results

(A) Spherulites

When a concentrated suspension of fd (15 mg/ml or greater) is stored for several days, it may separate into two phases, an upper

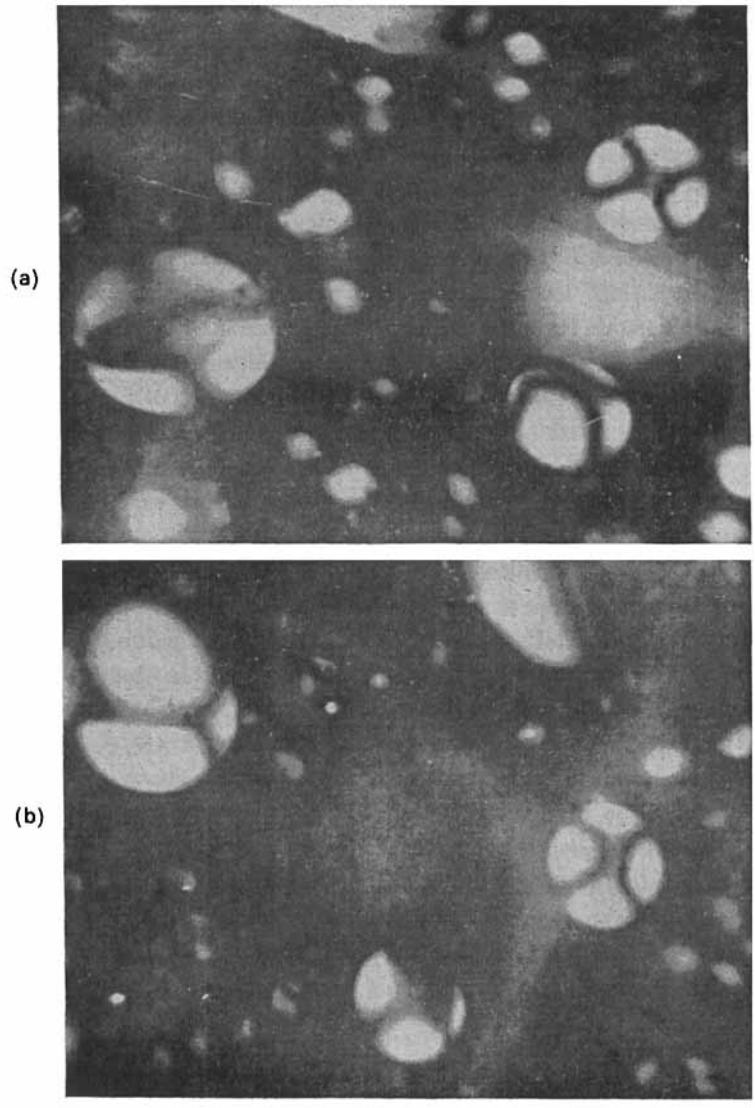


Figure 1. Spherulites of fd. A 16 mg/ml suspension of fd in 0.01 M Na_2HPO_4, pH 12 was stored for several days. A lower birefringent phase appeared, having a concentration of 19 mg/ml. When the lower phase was disturbed, spherulites and tactoids appeared in the isotropic phase. A sample of this phase, containing both spherulites and tactoids, was placed in a depression in a glass slide, and examined at a magnification of 130 × under crossed polars (a). The appearance of the dark cross in the spherulites altered when the specimen was rotated by about 40°, still between crossed polars (b).

isotropic phase and a lower birefringent phase, as found for other viruses.[1,11] The lower phase contains fewer impurities, as judged by the ultraviolet spectrum.[9] The concentration at which separation occurs agrees qualitatively with the equation derived by Flory[5] for phase separation in suspensions of rod-shaped molecules.

If the birefringent phase is mechanically disturbed, small tactoids appear, embedded in the amorphous matrix; these grow into spherulites (Fig. 1). Under crossed polars, these spherulites show a black cross with its arms parallel to the planes of polarization of the two polars. The larger index of refraction is tangential to the surface of the spherulite. Since the intrinsic birefringence of fd is positive,[8] the molecules must lie with their long axes parallel to the surface of the spherulites, as observed for α-helical polyaminoacids[16] and ribonucleic acid.[18] On standing, these spherulites coalesce and drift downwards, forming the dense birefringent bottom phase.

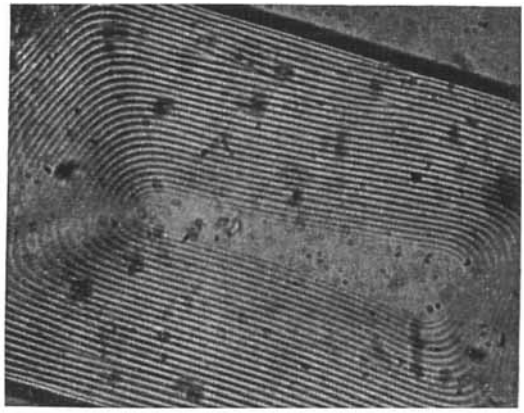
" Deformed spherulites " are seen when concentrated gels of fd are stored in sealed quartz capillaries (Fig. 2). These structures completely fill the capillary and are therefore constrained to have its shape. However these structures show many of the characteristics of spherulites. Equidistant parallel lines are seen without crossed polars (Fig. 2a); with one polar, the lines which are perpendicular to the plane of polarization disappear, as found for other spherulites.[12] A " radial line of disinclination "[16] is seen at a specific depth of focus in the spherulite (Fig. 2c).

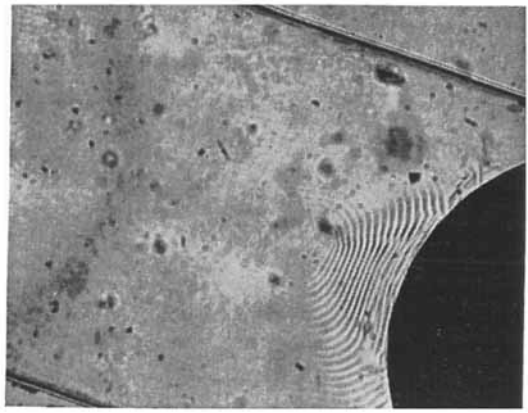
The spacing of the lines varies with pH and ionic strength, and decreases with increasing concentration. Some representative values are: in a " dry gel " (about 60% w/w fd), the spacing is 1 μ; in a 10% gel, about 15 μ; in a 2% gel, about 90 μ.

(B) Optical Rotation

In a cholesteric liquid crystal, the molecules are arranged in sheets, with their long axes parallel. These sheets are stacked one above the other, with a small regular rotation between the direction of alignment in each sheet and the one above (see, for example, Fig. 1 of Ref. 16). The parallel lines seen in spherulites are spaced at half the pitch of the helix followed by these sheets, seen edgeways-on.[2,4,14,16,17]

The centers of the " deformed spherulites " which fill the capillaries are not always completely filled with parallel lines (Fig. 2a). In these





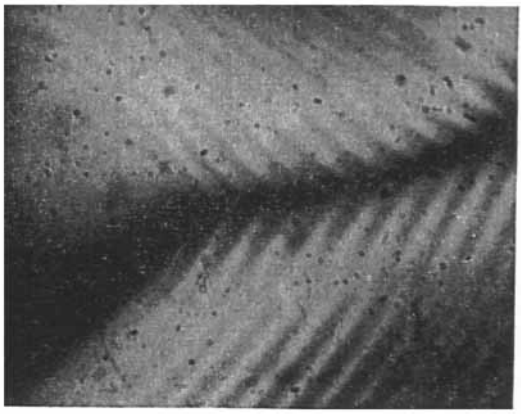


Figure 2. Distorted spherulites. A 1% gel of fd at pH 7 was withdrawn into a quartz capillary, diameter 0.8 mm, and stored. After several days, a distorted spherulite structure appeared, which showed dark parallel lines even in unpolarized light (a, 60 × magnification). When the same structure was viewed through one polar, with the plane of polarization perpendicular to the long axis of the capillary, the parallel lines perpendicular to the plane of polarization disappeared (b, 60 × magnification). A radial line of disinclination was seen at one point in the structure (c, 300 × magnification).

central regions, the line of sight is perpendicular to the sheets of molecules. These regions show large form optical rotation, because of the rotation of these stacked sheets about the line of sight (Fig. 3).

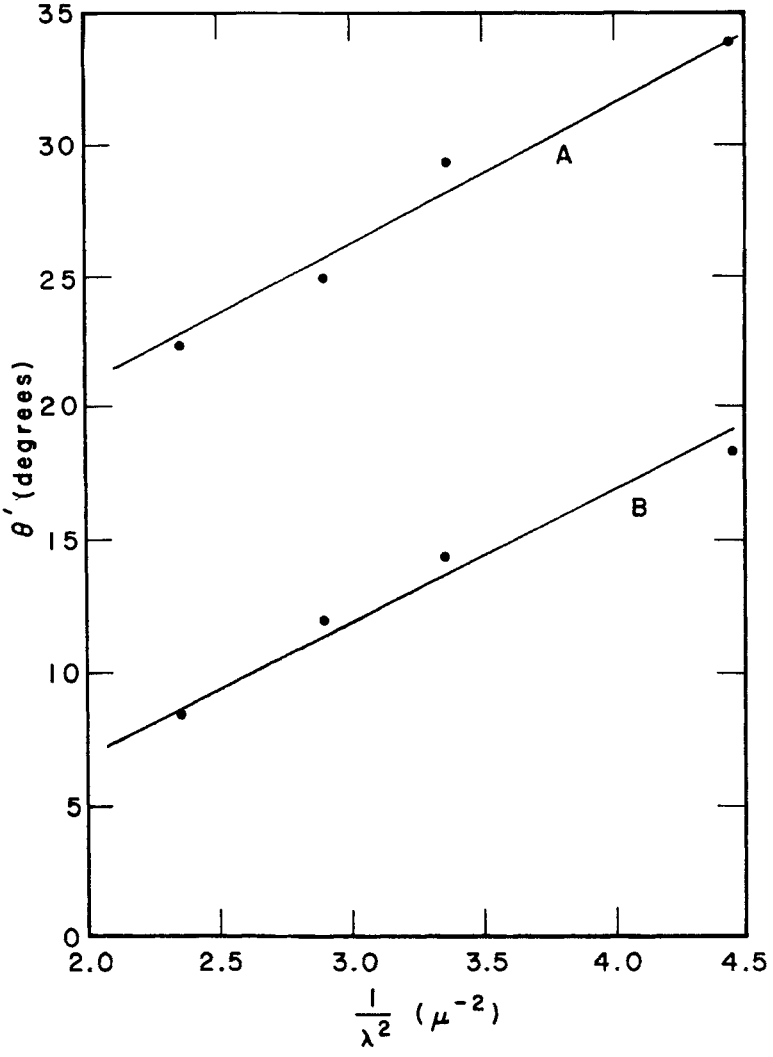


Figure 3. Optical rotation as a function of wavelength. The apparent optical rotation, θ', was measured in the center of distorted spherulites which had formed in a 10% gel in a capillary. A: the specimen shown in Fig. 2, 0.7 mm thick; spacing between dark lines in the spherulite (P) is 28 μ. B: another specimen, 0.3 mm thick, P = 34 μ.

The true optical rotation θ is related to the measured rotation θ' by

$$\theta = \frac{2k\pi \pm \theta'}{t} \tag{1}$$

where k is an integer, and t is the thickness of the specimen. If the pitch of the twisted structure is large compared with the wavelength of the incident radiation, the de Vries equation[4] for optical rotation may be approximated by[16]

$$\theta = -4.5 \times 10^4 \times \frac{Pn^2}{\lambda^2} \frac{\text{degrees}}{\mu} \tag{2}$$

where P is the pitch of the helix and n is the birefringence of the untwisted material for wavelength λ. Figure 3 shows that θ' varies linearly with $1/\lambda^2$, as predicted by the modified de Vries equation.

Since neither k nor t in Eq. (1) varies with λ, $d\theta/d(1/\lambda^2) = d\theta'/d(1/\lambda^2)$, and the slope of the lines in Fig. 3 can be used to calculate the birefringence, $n = 0.001$. The concentration of these gels is 10% w/v. Fibers of fd, which have a concentration of 90% w/v at 66% relative humidity, have a birefringence of 0.005 to 0.01.[8] Therefore the specific birefringence calculated from optical rotation agrees with the direct measurements of birefringence on fibers.

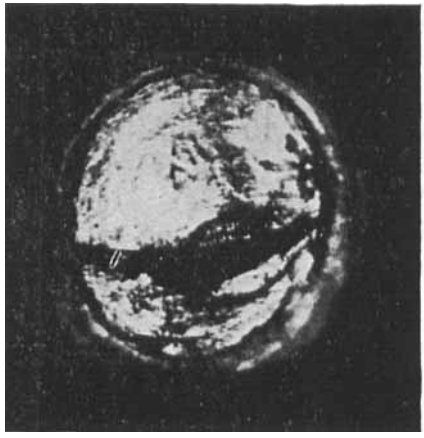


Figure 4. Gel dried in a capillary, viewed along the capillary axis, between crossed polars. The X-ray diffraction pattern of such a specimen, when the X-ray beam is perpendicular to the capillary axis, is shown in Plate II of Ref. 8. The specimen is cracked across the middle.

In some specimens of fd which have dried in a quartz capillary, the long axes of the virions lie in planes perpendicular to the long axis of the capillary, but the planes themselves show no preferred orientation.[8] When such specimens are viewed down the axis of the capillary between crossed polars, they are illuminated (Fig. 4). Local regions of the specimen show extinction when one of the polars is rotated. Therefore these specimens behave as if there is a small regular rotation between the direction of alignment of molecules in each sheet and the one above, as one moves along the axis of the capillary: the same optical rotation effect seen for more dilute suspensions. These specimens show iridescent colors when viewed perpendicular to the axis of the capillary.

(C) Iridescent Gels

As a gel of fd dries, the spacing between the dark lines in the gel decreases from tens of microns (Fig. 2a) to about 1 μ (Fig. 5). These

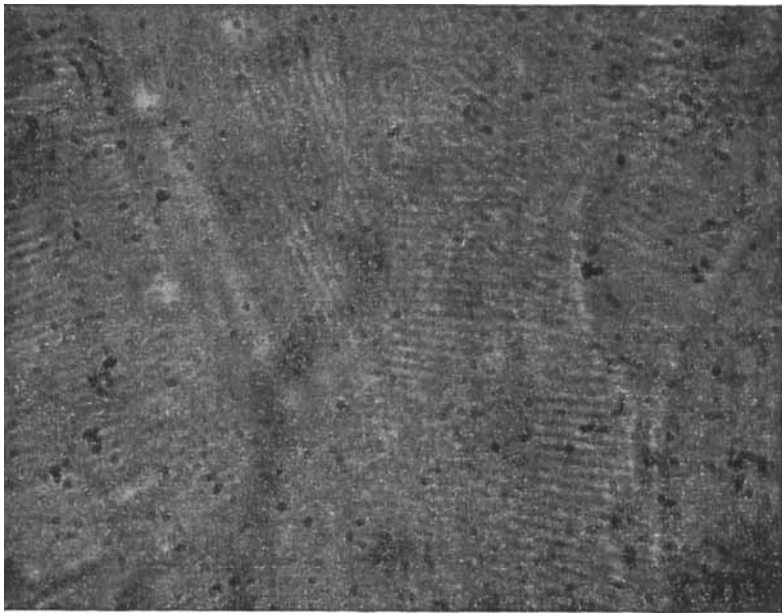


Figure 5. An iridescent region from a dry fd specimen in 0.01 M Na_2PO_4, pH 12, was viewed under 1200 × magnification. Fine parallel lines were seen, separated by about 1.2 μ.

concentrated gels show iridescent colors when illuminated with a beam of white light (Fig. 6a). The planes act as a diffraction grating for light (Fig. 6b, c; Table 1).

TABLE 1 Diffraction of Visible Light by Iridescent Gels of fd

λ (nm)	2θ (degrees)†	d (nm)‡
435	27.5	686
546	34.5	692
578	37.5	675

† The estimated uncertainty in measuring 2θ is $\pm 1°$.
‡ $d = \lambda/2n \sin\theta$, where $n = 1.33$ is the index of refraction of light in the gel, and θ is half the angle between the incident and diffracted beams.

Such iridescent gels are also observed if a fiber of fd is sealed in a capillary with distilled water, and allowed to swell. The distribution of intensity over the X-ray diffraction pattern of such a swollen fiber is similar to the distribution of intensity for dry fibers.[8]

In one experiment, a wet gel which showed dark parallel lines similar to those in Fig. 2a was allowed to dry, and showed iridescent colors; the sample was then re-wet, and the parallel lines re-appeared. The transition between the two kinds of liquid-crystal forms is reversible.

(D) Twisted Ribbons

Some gels which were formed in capillaries showed structures which appear like ribbons twisting around the inner surface of the capillary wall (Fig. 7). These structures might possibly be related to the cybotactic nematic phase of liquid crystals,[3] or may be due to another cause.

4. Discussion

Liquid crystalline behavior has been seen for other viruses[1,6,11] and has been extensively studied for poly α-amino acids.[12–16] However fd virus appears to be one of the most complicated molecules yet reported which shows cholesteric liquid crystal behavior. Further

investigation of this phenomenon may shed light on the long-range forces acting between biological macromolecules.

Acknowledgement

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