

Cholesteric Phase in Virus Suspensions

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We report measurements of the cholesteric pitch and twist elastic constant (K_{22}) in monodisperse suspensions of the rodlike virus filamentous bacteriophage *fd*. Measurements were taken for concentrations spanning the entire cholesteric region at several ionic strengths and temperatures. In the limit of high ionic strength the cholesteric pitch (P_0) scales with concentration (c) as $P_0 \propto c^{-1.66}$. As the ionic strength decreases, the scaling exponent systematically changes to lower values.

Introduction

The system with the simplest intermolecular interaction known to exhibit all the essential features of the nematic state is that of a hard rod suspension.^{1,2} Because of its inherent simplicity, much effort has been put into understanding the relationship between the microscopic parameters of hard rods and the resulting liquid crystalline behavior at the macroscopic level. In nature it often happens that a symmetry of the nematic phase can be reduced to form a cholesteric phase, where the nematic director follows a helical path in space. Formation of such a phase at the macroscopic level is usually associated with chirality of molecules at the molecular level. Details of how a simple change of a few atomic positions, required to make a molecule chiral, causes a drastic change in self-organization at the macroscopic level remain unknown. However, in the continuum limit, where details of the microscopic interactions are ignored, formation of the cholesteric phase is understood as a competition between two elastic energies. On one hand, the free energy of a chiral nematic is lowered in a twisted state because of the torque a chiral molecule exerts on its neighbor. Such a contribution to the free energy is characterized by the “twist” constant K_t . On the other hand, creation of an elastically distorted state characterized by the usual twist elastic constant K_{22} raises the free energy.³ It follows that the wavelength of the cholesteric pitch is proportional to the ratio of K_t/K_{22} . At present, the challenge lies in calculating the value of the “twist” constant K_t for a given molecule with known microscopic interactions.

Inspired by work of Onsager, Straley made the first attempt to explain the microscopic origin of the cholesteric phase.^{4,5} He considered rods with threads of definite handedness and for the first time obtained an expression for K_t as a function of the microscopic parameters of a threaded rod. As in the case of Onsager’s hard rods, Straley’s cholesteric phase is purely entropy driven. The nonzero value of the chiral “twist” constant K_t is associated with the free volume gained as threaded rods approach each other at an well-defined angle. This work was later extended to account for flexibility of rods.^{6,7}

It is not clear if there are lyotropic liquid crystals where the cholesteric phase is purely entropy driven and therefore most of the predictions of the Straley model remain untested. An alternative proposal for the origin of a nonzero K_t constant involves chiral attractions of van der Waals origin.⁸ It is likely that for almost all experimental systems both entropic temperature-independent interactions and attractive temperature-dependent interactions contribute to the cholesteric twist, further complicating the problem. Harris and co-workers noticed that if the threaded rod is allowed to rotate freely around its long axis, chirality will be effectively averaged away and proposed that short-ranged biaxial correlations are critical for formation of a cholesteric phase.^{9,10} The implication of their work is that all mean-field theories, like the one of Straley, do not capture the essence of cholesteric phase since they ignore all correlations.

Bacteriophage *fd* is a chiral, monodisperse rodlike colloid that forms a cholesteric phase with a characteristic “fingerprint” texture shown in Figure 1a.¹¹ *fd* has a contour length of 880 nm,¹² persistence length of 2200 nm,¹³ and a linear charge density of $2e^-/\text{\AA}$ at pH 8.1.¹⁴ However, Pf1, a chiral virus with a structure extremely similar to *fd*,^{15,16} does not show any evidence of forming a cholesteric liquid crystal as shown by the absence of a “fingerprint” texture in Figure 1b. This sets the lower limit of the cholesteric pitch of Pf1 virus to the size of the capillary. *The theoretical challenge is to explain why two such similar chiral molecules have extremely different values of the cholesteric pitch.*

The concentrations of the coexisting isotropic and cholesteric phases are quantitatively explained by the Onsager theory establishing *fd* as an ideal model of hard rods.^{17,18} On the other hand, the origin and mechanism of

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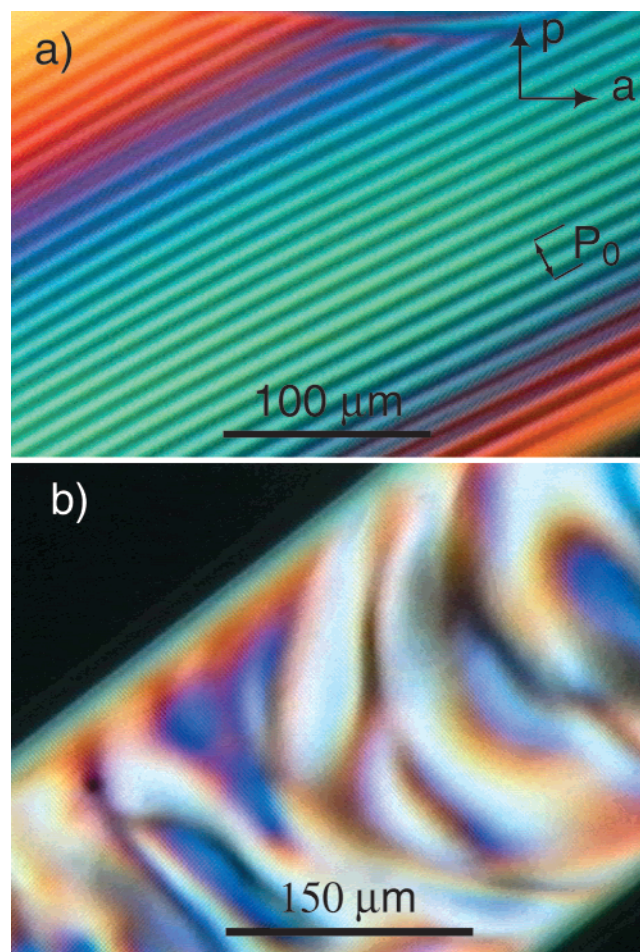


Figure 1. Typical texture of a cholesteric phase of a liquid crystalline sample of *fd* observed with polarization microscopy. Dark lines correspond to regions where the rods are perpendicular to the plane of the paper and bright lines correspond to regions where rods are in the plane of paper. The length of the cholesteric pitch (P_0) spans two bright and two dark lines as indicated in the micrograph. The concentration of the *fd* virus is 48 mg/mL and the ionic strength is 8 mM. The positions of the polarizer (p) and analyzer (a) are indicated in the upper right corner. (b) Texture observed with a polarization microscope for a nematic phase formed by filamentous virus Pf1. A lower limit of the pitch of Pf1 is the capillary diameter of 0.3 mm. Although at the molecular level the two viruses have remarkable similarity, there is no evidence of a cholesteric phase in Pf1 as indicated by lack of a fingerprint texture. The sample concentration is 38 mg/mL and the ionic strength is 8 mM.

the formation of the cholesteric structure in liquid crystalline *fd* remains a challenge. It is important to note that the Onsager theory predicts equally well the concentration dependence of the isotropic–nematic and isotropic–cholesteric first-order phase transition. The reason for this is that the free energy difference between the isotropic and nematic phase is much larger than the free energy difference between the nematic and cholesteric phase. The average twist in the cholesteric phase between two neighboring molecules is generally less than 0.1° . This is negligible when compared to the magnitude of director fluctuations of rodlike molecules in the nematic phase, which are typically around 10° .

In this paper we study in detail the continuum properties of the cholesteric phase formed by *fd* virus. We measure

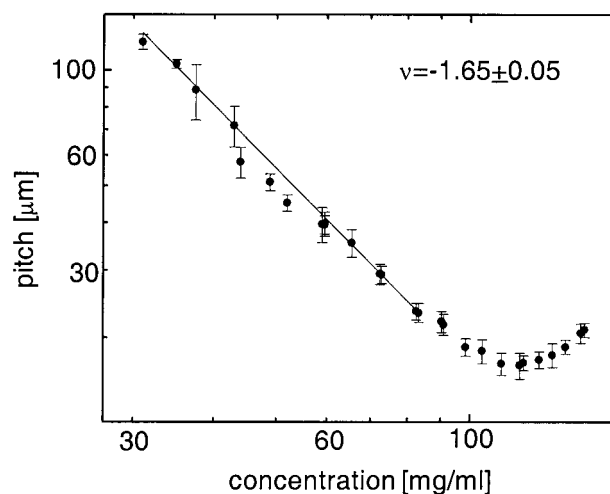


Figure 2. log–log plot of the cholesteric pitch (P_0) versus concentration (c) of the *fd* virus. The ionic strength was 53 mM. A fit to $P_0 \propto c^v$ reveals the scaling exponent to be -1.65 ± 0.05 . The data spans the entire range of concentrations of the cholesteric phase for this ionic strength and temperature. The sample is isotropic below 30 mg/mL and smectic above 150 mg/mL.

the dependence of equilibrium pitch on concentration and ionic strength and compare it to theory. For two ionic strengths we also measure the value of the twist elastic constant (K_{22}) as a function of concentration. Initial studies of the cholesteric phase of *fd* can be found in the thesis of Oldenburg.¹⁹

II. Experimental Results

Bacteriophage *fd* was grown and purified using standard techniques of molecular biology.²⁰ A stock solution at a volume fraction of 5% was dialyzed at room temperature against Tris-HCl buffer at pH 8.1 of the desired ionic strength and 3 mM sodium azide (NaN_3) were added to prevent any bacterial growth. This solution was spun in an ultracentrifuge which resulted in very viscous iridescent pellet indicating a smectic or crystalline order. The pellet was resuspended at 4°C overnight in the amount of buffer so that its final concentration was just above the cholesteric–smectic transition.²³ A dilution series was made from the smectic to isotropic phase. The concentration was measured using the extinction coefficient $\text{OD}_{269\text{nm}}^{\text{1cm}} = 3.84$.¹⁸ Quartz X-ray capillaries of 0.7 mm diameter were cleaned with sulfuric acid and repeatedly rinsed with deionized water before being filled with *fd* samples. After 24–48 h the sample would equilibrate and a typical cholesteric texture was observed. As described previously, a *fd* suspension exhibits an increase in the isotropic–cholesteric concentration over a period of few weeks.²¹ The origin of this time dependence is not known (although we suspect bacterial growth). We also observed that the cholesteric pitch systematically increases over the same time period. Typically the values of the coexistence concentrations shift by 1% per week for first few months after the sample is prepared.²² Because of this effect we performed measurements on samples that were at most a few days old.

When viewed under a polarizing microscope the cholesteric phase displays typical dark and bright stripes indicating that molecules are perpendicular and parallel to the plane of polarizers, respectively as is shown in Figure 1. The distance between two bright lines is equivalent to half the value of the pitch P_0 . The microscope objective was focused on the midplane of the cylindrical capillary, and the picture was displayed on a

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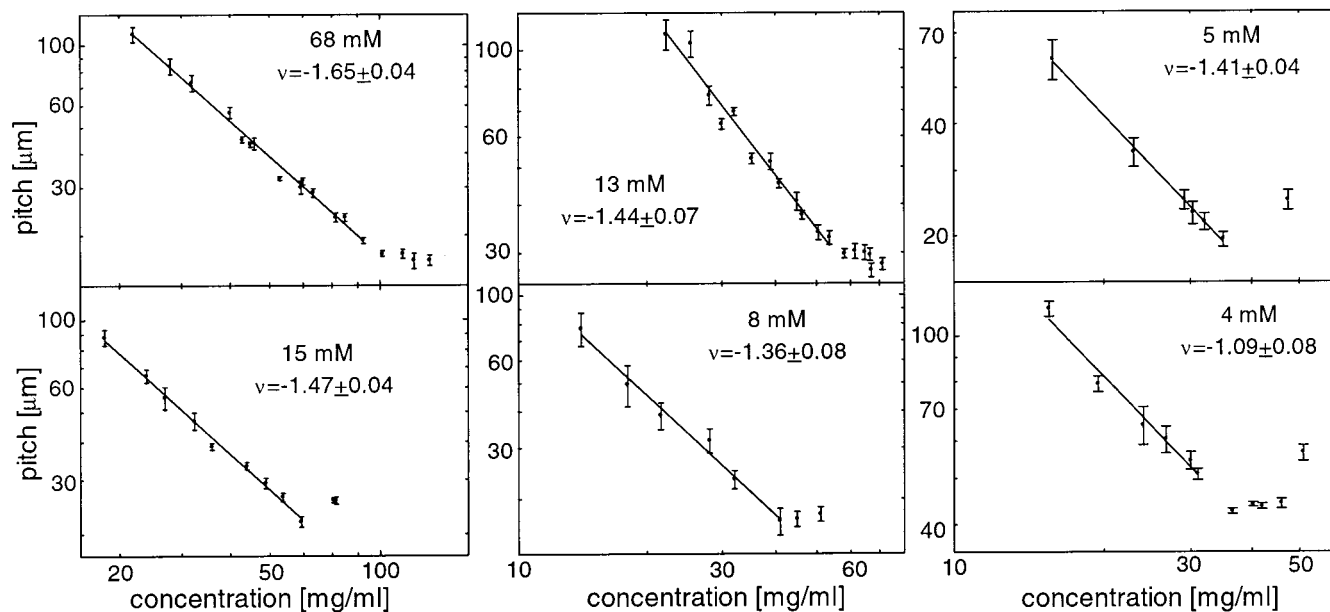


Figure 3. log–log plots of the cholesteric pitch dependence (P_0) versus fd concentration (c) for six different ionic strengths ranging from 68 to 4 mM. In each plot data were fitted to an equation of form $P_0 \propto c^\nu$. At the highest ionic strength the exponent ν equals -1.65 ± 0.04 and systematically decreases with decreasing ionic strength until it reaches the value of -1.09 ± 0.08 for the lowest ionic strength of 4 mM. Each data set spans the entire cholesteric phase from the isotropic boundary at low concentrations to the smectic phase at high concentrations.

video monitor where the cholesteric pitch was measured using a ruler. Measurements of pitch are very sensitive to defect and boundary conditions and can vary by as much as 20% within the same sample. However, by repeating the measurement 15–25 times along the full length of the sample, it is possible to get a reproducible value of the cholesteric pitch.

As the concentration of fd is increased, we observe a first-order isotropic to cholesteric transition,¹⁷ which is followed by a first-order cholesteric to smectic phase.²³ We measured the pitch of the cholesteric phase for concentrations ranging from the isotropic to the smectic phase. The typical behavior of cholesteric pitch as a function of concentration is shown in Figure 2. We observe that the pitch decreases with increasing concentration until it saturates at a certain value. As the concentration of the virus increases further and approaches the smectic transition, we observe a slight unwinding of the pitch. This unwinding of the cholesteric pitch close to the smectic transition has been observed before in thermotropic cholesteric liquid crystals.²⁴ It is generally assumed that it is due to presmectic fluctuations in the cholesteric. Curiously, when we looked for these presmectic fluctuations with light scattering, we did not observe any,²³ even though other virus liquid crystals (TMV) do show strong presmectic fluctuations.²⁵ At the cholesteric–smectic phase boundary the cholesteric pitch abruptly unwinds. We repeated the measurements for a range of ionic strengths from 4 to 63 mM as shown in Figure 3.

By measuring the critical magnetic field necessary to induce the cholesteric to nematic phase transition, it is possible to deduce the value of the twist elastic constant.^{3,26,27} The following formula (in cgs units) relates the critical field to the value of the twist elastic constant K_{22}

$$H_c = \frac{\pi}{2} \left(\frac{K_{22}}{\chi_v} \right)^{1/2} q_0 = \pi^2 \left(\frac{K_{22}}{\chi_v} \right)^{1/2} \frac{1}{P_0} \quad (1)$$

The value of the diamagnetic anisotropy $\chi_0 = \chi_{||} - \chi_{\perp}$ per molecule for fd is 7×10^{-25} erg/ G^2 in cgs units.²⁸ To convert χ_0

to the diamagnetic anisotropy per unit volume χ_v used in eq 1, it is necessary to multiply χ_0 with the number of fd molecules per 1 cm^3 (n) given by $n = cN_A/M_W$, where c is the mass concentration of fd , $M_W = 1.64 \times 10^7 \text{ g/M}^{18}$ is the molecular weight of fd , and N_A is Avogadro's number. We note that the reported value of χ_0 used in this paper,²⁸ is an overestimate (by at most a factor of 2) of the actual value.²⁹

We placed the sample with the long axis of the cylindrical capillary parallel to the magnetic field and simultaneously observed the unwinding of the cholesteric pitch under a microscope. Theoretically we expect that the equilibrium pitch will scale as the fourth power of applied field. Utilizing this sharp dependence on magnetic field, we measured the highest value of the field at which the characteristic fingerprint cholesteric texture could still be observed and the lowest field at which we observed no cholesteric texture. Using eq 1 we can calculate the range within which we expect the true value of twist elastic constant. We have measured the value of the twist elastic constant for various fd concentrations at two ionic strengths of 68 and 13 mM as is shown in Figure 4.

The dependence of the cholesteric pitch on temperature is shown in Figure 5. For this part of the experiment phosphate buffer was used because of the smaller temperature dependence of pK_a . As the temperature is increased, we observe rapid unwinding of the cholesteric pitch. The measurements are reversible, and upon a sudden temperature quench the sample winds up within a few minutes as the cholesteric pitch attains its equilibrium value.

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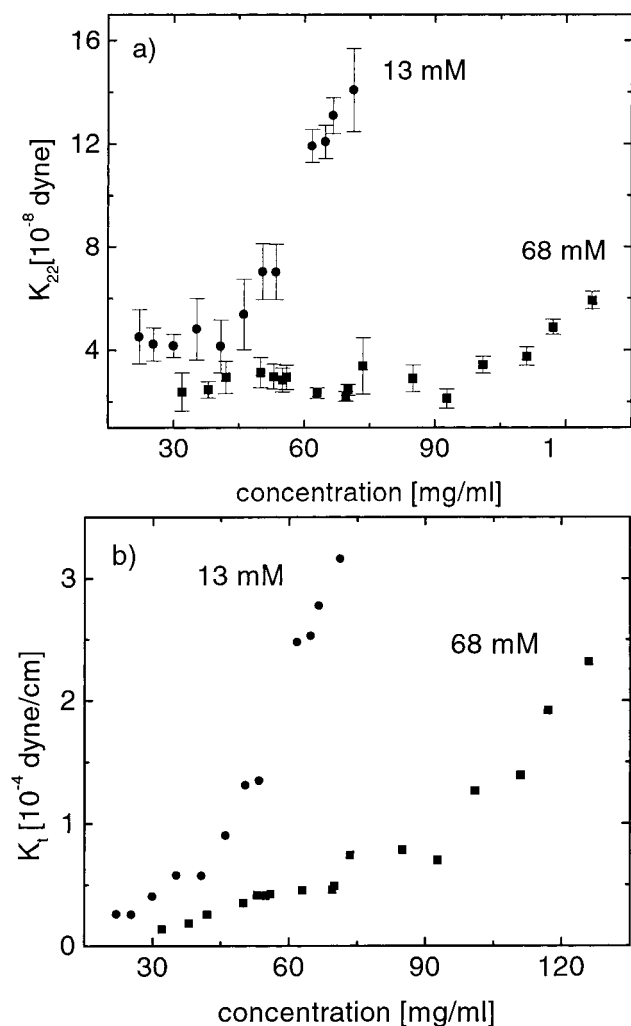


Figure 4. (a) Concentration dependence of the twist elastic constant (K_{22}) determined by measuring the critical magnetic field required for inducing the cholesteric to nematic transition at two ionic strengths of 13 and 68 mM. Error bars indicate a range between lowest magnetic field required to unwind the sample and the highest magnetic field at which there is still evidence of the cholesteric phase. (b) Chiral "twist" constant (K_t) calculated from the relation derived from continuum theory $K_t = \pi K_{22}/P_0$ for two ionic strengths of 13 to 68 mM. Note that the sample concentrations at higher ionic strength of 68 mM do not span the entire cholesteric range. The reason for this is that the accessible magnetic fields were not strong enough to completely unwind samples with very high concentration.

III. Discussion and Conclusion

Bacteriophage *fd*, as most other biological colloids, has a charged surface to maintain its stability in solution. Onsager was first to show that in the dilute limit the free energy of a charged rod is approximately equal to a free energy of a neutral rod with an effective diameter larger than its bare diameter.^{1,32} It follows that the volume fraction of the isotropic to nematic phase transition scales with D_{eff} . The dependence of D_{eff} on ionic strength for *fd* was calculated previously,¹⁷ and the concentration dependence of the coexisting isotropic–cholesteric phases as a function of ionic strength was previously investigated.¹⁷

Motivated by the predictions from various theories^{5–7} we have tried to fit our measurements of the pitch to an

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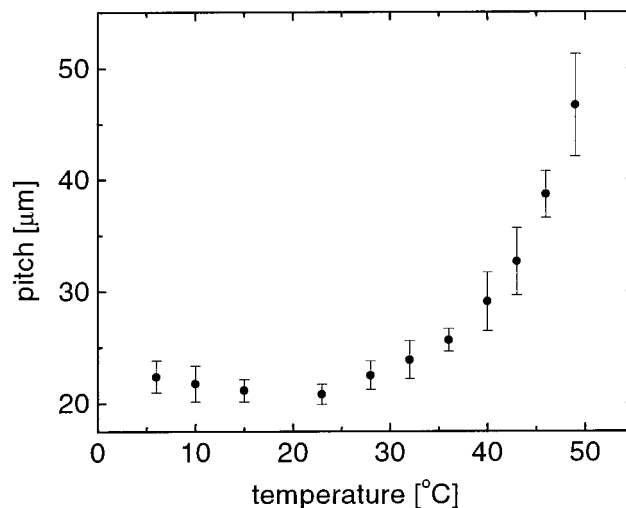


Figure 5. Dependence of *fd* cholesteric pitch on temperature. The rapid increase in pitch at high temperatures is not due to denaturing of the virus since the sample regains the original cholesteric structure upon cooling to room temperature. The concentration of the *fd* sample was 32.5 mg/mL and the ionic strength was 8 mM. The sample was far from the cholesteric–smectic phase boundary, and therefore the pitch unwinding cannot be due to presmectic fluctuations.

exponential form $P_0 \propto c^{-\nu}$ as shown in Figure 3, for ionic strengths from 4 to 68 mM. At 68 mM ionic strength we find that the scaling exponent ν has a value of 1.65 ± 0.05 . Physically, at high ionic strength the Debye screening length κ^{-1} becomes very small, and the effective diameter approaches the hard rod limit.¹⁷ However, even for 68 mM ionic strength, D_{eff} (13 nm) is still considerably larger than the bare diameter (6.6 nm). As the ionic strength decreases to 4 mM, D_{eff} increases to about 30 nm. Experimentally, we find that the exponent ν systematically decreases with decreasing ionic strength until it reaches the value of 1.09 ± 0.08 for low ionic strengths of 4 mM.

Other experimental investigations of lyotropic cholesteric liquid crystals involved the neutral synthetic polymer poly-benzyl-L-glutamate (PBLG),^{33,34} charged DNA,³⁵ and charged cellulose suspensions.³⁶ In the case of PBLG, the dependence of pitch on concentration scales with exponent $\nu = -1.8$. In the limit of high ionic strength we measure the value of the equivalent exponent for *fd* to be -1.65 . It might be expected that as ionic strength is further increased and *fd* increasingly becomes hard-rod-like, the agreement between PBLG and *fd* data would be even better. Experimental results for both *fd* and PBLG are close to the theoretical prediction for the scaling constants ν due to Odijk and Pelcovits, which are -1.66 and -2.0 , respectively.^{6,7}

For DNA and closely related synthetic double-stranded RNA there are conflicting reports in the literature. Jizuka et al.,³⁷ in their study of double-stranded RNA, reported the exponent to be -1.1 . Senechal et al.,³⁸ on the other hand, finds that the scaling exponent for an equivalent system to be -0.5 . One possible explanation for this

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discrepancy is the fact that the samples used had different ionic strength. Senechal et al. did their experiment in distilled water, while Jizuka's experiments were done at an ionic strength of 100 mM. This agrees with our observations about the influence of ionic strength on the scaling exponent. For DNA of contour length equal to the persistence length, Van Winkle reported that the cholesteric pitch is independent of the concentration.³⁵ However, it is also reported that DNA forms a pre-cholesteric phase with large cholesteric pitch and undetermined structure. This is in contrast to the phase behavior of fd where there is no sign of any transition between two cholesteric phases of different pitches.

Using a magnetic field induced cholesteric to nematic phase transition, we have measured the concentration dependence of the elastic constant K_{22} . Although the data are noisy, the value of K_{22} is almost constant in samples of low concentration. Once the concentration of the virus is close to the smectic phase concentration, the value of K_{22} rapidly increases. It is interesting to note that samples at low ionic strength have a significantly greater plateau value of K_{22} than the high ionic strength samples, in contradiction to the theory of elastic constants that takes into account the charge of rodlike particles.³⁹ Dupre³³ measured the value of the twist elastic constant in PBLG and found that there is little variation over the entire concentration range, which agrees with the plateau we observe in fd samples. However, in PBLG there is no sudden increase in K_{22} at high concentrations. Measuring values of the twist elastic constant (K_{22}) and the pitch for identical samples enabled us to obtain values of the chiral twist constant K_t using the relation $P_0 = 2\pi K_{22}/K_t$,¹⁰ as shown in Figure 4.

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Sato⁴⁰ and co-workers have found that the expression for the cholesteric pitch splits into an entropic and enthalpic part. The entropic part, due to hard core repulsive forces, is temperature independent, while the enthalpic part, due to attractive forces, scales inversely with temperature. They measured the temperature dependence of the cholesteric pitch for a number of liquid crystalline polymers and found that the pitch scales with inverse temperature. It is important to stress that their analysis is thermodynamic and therefore disregards all complex details of molecular interactions. Surprisingly, we find that the temperature dependence of the cholesteric pitch of fd does not agree with this general thermodynamic formalism. A possible source of this discrepancy lies in the assumption used by Sato et al. that changing temperature has no effect on the internal structure of the polymer. While this is true for most synthetic polymers, it is well-known not to be the case for biopolymers. Viruses Pf1 and M13 undergo structural symmetry transitions upon decreasing temperature below 8 °C and changing surface charge, respectively.⁴¹ There are also indications that the flexibility of fd depends nonmonotonically on temperature.²² Any one of these factors could be the reason for the unusual dependence of pitch on temperature.

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