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Liquid crystal phase behavior of a DNA dodecamer and Sunset Yellow

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Liquid crystal phase behavior of a DNA dodecamer and Sunset Yellow

by

Joseph George Theis

A thesis submitted to the Faculty of the Honors Program of the University of Colorado in partial fulfillment of the requirements for the degree of Bachelor of Science with Honors Department of Physics

Defended October 12, 2017

Examining Committee: Prof. Noel Clark, Department of Physics (Thesis Advisor)
Prof. John Cumalat, Department of Physics, Prof. David Walba, Department of Chemistry
The organic molecule Sunset Yellow, a chromonic dye, and reverse Dickerson dodecamer DNA, a self-complementary, 12 base pair strand of nucleic acids, both self-assemble into rod shaped aggregates that exhibit liquid crystal phases in solution. The sunset yellow molecules and the nano-DNA duplexes have similar structure with hydrophobic cores and peripheral hydrophilic ions. The focus of this research is on mixtures of these two aggregates in miscible liquid crystal states and the phase separation that occurs at higher concentrations in the columnar phase. The structure and phase space of this mixture was determined using polarized optical microscopy and x-ray diffraction. In the columnar phase, these mixtures provide experimental data on the two-dimensional arrangement of binary, hard disks. Moreover, studying these mixtures will further the understanding of polymer, liquid crystal mixtures. Our results demonstrate selectivity in DNA aggregation, supporting the hypothesis that liquid crystal ordering auto-catalyzed the polymerization of nucleic acids before the biological mechanisms responsible for assembly existed.
Acknowledgements

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Chapter 1

Introduction

The work presented here is an extension of research conducted by Nakata [1], Smith [2], and Yamaguchi [3], who studied deoxyribonucleic acid (DNA) liquid crystals (LCs) and their mixing with various chromonic dyes. Most chromonic dyes are immiscible with DNA and form distinct, separated phases across the LC range. Smith and Yi discovered that sunset yellow (SSY), a chromonic dye, was highly miscible with DNA. This made these mixtures of specific interest. The LC phases of pure SSY have been well studied by Edwards [4] and Park [5]. Their results, namely the phase diagrams and structures are compared to the results we obtained for pure SSY in Section 2.3. Nakata conducted the seminal work for DNA liquid crystals, establishing phases for oligomers ranging from 6 to 20 base pairs [1]. LC phases have also been observed for the 12 base pair reverse Dickerson dodecamer (rDD). The phases and structure of rDD were described by Smith [2] and Nakata [1]. Our results with pure rDD phases will be discussed in Section 2.4. The mixture of rDD and SSY in LC phases is the focus of this research. These mixtures have implications on the origin of DNA/RNA and our understanding of the phase behavior of 2D binary disk mixtures.

1.1 Liquid Crystals

 Liquids are characterized by their ability to flow, the isotropic disorder of the molecules, and their near-incompressibility. Solids do not flow, as the atoms are bound together in either a lattice or amorphous structure, thus they maintain their shape and volume. Liquid crystal
is a mesophase that some materials exhibit between the liquid and solid states. Liquid crystals have some form of structural anisotropy, but can still flow in at least one spatial dimension. This anisotropy is either in a preferred orientation of the constituent species or in some long-range positional ordering, a lattice with at least one translational degree of freedom.

In order to form these anisotropic LC phases, the species dissolved in solution must have a certain degree of anisotropy themselves. Onsager showed in the 1940s, that thin, hard spherocylinders will form a nematic phase at sufficient densities due to transitions driven by entropy. The transition from isotropic fluid to nematic LC is shown in Figure 1.1. Structural order increasing with increasing entropy is a counter intuitive notion. However, Onsager’s model demonstrated that at a critical density the loss of entropy associated with orientational ordering is less than the increase in entropy associated with the decreased excluded volume. Therefore, Gibb’s free energy, given in Equation 1.1 will be minimized for the anisotropic phase. This reduction in excluded volume drives other LC phase transitions, specifically the nematic-columnar transition, which is relevant to this work. The parameter quantifying orientational order is given in Equation 1.2, with order parameter $S$ ($S$ is the entropy in Equation 1.1), and $\theta$, shown in Figure 1.1

$$G(p, T) = H - TS$$ \hspace{1cm} (1.1)

$$S = \langle \frac{3 \cos^2 \theta - 1}{2} \rangle$$ \hspace{1cm} (1.2)

Onsager considered spherocylinders, with length $L$ and and diameter $D$, in the limiting case where $L/D \gg 1$. Frenkel and Bolhius used Monte Carlo simulations to find that the isotropic to nematic transition would occur for spherocylinders with $L/D > 4.7$. These anisotropic species can be a single molecule, like the long molecule CB7CB, or an aggregation of smaller molecules into a rod that satisfies the L/D ratio. The latter is the source of the anistropy in the case of SSY and rDD.

The nematic phase (N) has orientational order but the molecules are positioned ran-
domly. This is the lowest order LC phase and is the least viscous. In a specific nematic phase, called cholesteric or chiral nematic (N*), the nematic director, denoted n in Figure 1.1, rotates between planes of aligned aggregates. This phase will be discussed in Section 2.4. The other LC phase relevant to this work is columnar phase (C). This phase occurs at lower temperatures/higher concentrations and has both orientational and positional ordering. The aggregates align nearly parallel, in a 2D hexagonal columnar lattice shown in Figure 1.1. This phase is not a solid because these columns can still slide past one another in the direction of the director.

Figure 1.1: The transition from an isotropic fluid, to the nematic, and then columnar LC phases with increasing order moving right. The columnar phase has positional and orientational order, while the nematic phase only has orientational order. n is the nematic director. \( \theta \) is the angle between the director and the molecules long axis. \( h_s \) is the stacking height between disks. \( d_c \) is the distance between columns in the columnar phase.

1.2 Origin of Life: Liquid Crystal Hypothesis

All known life uses nucleic acid polymers (DNA and RNA) to store information and pass information between generations. Despite the relevance of these polymers, their origin remains unknown. Cells and humans have developed complex methods to synthesize
and copy DNA. The question remains of how these biopolymers were generated before the complex biological processes existed.

Nakata demonstrated that complementary single-stranded DNA oligomers will bond to form double helices which form LC phases at high concentrations in water [1]. This was shown for strands as short as 6 nucleotides. Given the dimensions of duplexed DNA, it would require approximately 28 base pairs (BP) to satisfy the L/D > 4.7. Therefore, the 6 BP helices were stacking end-to-end to form rods of at least 30 BP. This work provided a potential mechanism for the formation of primordial DNA. The condensation and ordering of the liquid crystal phases may have provided the autocatalytic link for the polymerization of DNA. Furthering this hypothesis, it has recently been demonstrated that single nucleotides in solution with their complement are capable of forming LC phases [2]. This work is ongoing. A 2 BP sequence containing the 4 nucleotides is shown in Figure 1.2.

This work, while not directly probing this hypothesis, is a study of DNA liquid crystals and their selectivity in aggregation. By mixing molecules of similar structure and size with DNA LCs, we can explore the propensity of DNA to aggregate into homogeneous duplexed rods. This selectivity would facilitate the formation of these biopolymers in a highly concentrated “primordial soup,” where other organic molecules could potentially occupy interstitial sites in the rods, rendering them useless.
Figure 1.2: A 2 base pair sequence of DNA containing all four bases. Notice the aromatic rings of the nucleobases and the negative charge on the phosphate groups. The dotted lines represent the hydrogen bonds between the bases. Source: UFRGS
1.3 2D Binary Disk Mixtures

Many LC forming molecules are approximated well as hard spherocylinders or, in two dimensions, as hard disks. The term hard means that the volume they occupy is fixed and no two molecules can intersect. For charged species, such as SSY and DNA, which carry a charge of -2e per molecule or base pair, respectively, the effective radius of the hard shape can be extended according to the Debye screening of the charges in solution. For identical rods or disks the nematic and hexagonal-columnar ordering predictably minimize the free energy. For mixtures of spherocylinders of different lengths and radii (polydisperse mixture) the arrangement of the species is more complicated.

For the case of rDD-SSY mixtures, the lengths are polydisperse due to the unknown stacking length of the aggregates, and the radii are binary, either 20 Å for DNA or 13.4 Å for SSY. The ratio of these radii is .67. If we only consider minimizing the excluded area in a 2D columnar lattice, we can reduce the problem to pure geometry by considering the maximum density of packing for a binary distribution of circles. Likos and Henley investigated this for the whole spectrum of radii ratios [9]. Two lattices near the ratio of our mixture our shown in Figure 1.3. For ratios greater than .5 the hexagonal columnar lattice is always comparable to packing to the more complex lattices.

Mixtures of SSY and rDD show a coexistence of two phases at concentrations above/temperatures below those of a mixed columnar phase. This transition is governed by the competition between the entropy of mixing, given in Equation 1.3, and the reduced excluded volume gained from the separation. Here, R is the gas constant, and x is the mole fraction of one of the molecules. This equation is maximized at x=.5 for identical particles.

\[ \Delta S_{\text{mixing}} = -R \left[ x \ln x + (1 - x) \ln (1 - x) \right] \] (1.3)

For this phase separation, there is an upper critical solution temperature \( T_c \), above which all solutions show one phase, and a mass fraction \( \left( \frac{m_1}{m_1 + m_2} \right) \) where the transition from one phase to two phases is continuous \( (c_o) \), shown in Figure 1.4 as a molar fraction. For
Figure 1.3: 2D lattices for binary disk mixtures of two different radius ratios near that of SSY/rDD (0.67). Lattices selected on basis of maximal areal coverage. Source: Likos & Henley[9]
solutions that fall above this curve, there will be one phase. For solutions below, there will be two phases with mass ratios defined by the intersection of the specified temperature and the curve. From the molar ratios of the two states \((a,b)\) and the overall molar ratio \((c_i)\), the percent of the total solute in each phase can be determined by the lever rule given in Equation 1.4. \(\chi_a\) is the fraction of the total mass in the phase with molar ratio \((a)\).

\[
\chi_a = \frac{c_i - b}{a - b}
\]  

(1.4)
Chapter 2

Materials and Methods

This research was conducted in the Soft Materials Research Center (SMRC) at the University of Colorado Boulder. To supplement the in-house x-ray scattering experiments, additional x-ray diffraction measurements were performed at the Advanced Light Source at Lawrence Berkeley National Laboratory.

The primary means of observing these birefringent phases was polarized optical microscopy (POM). With a temperature controlled stage, this technique filled in much of the phase diagram and helped to inform our understanding of the structure of the material. It also allowed us to determine which locations in the three dimensional phase space (ratio of species, concentration, temperature) to probe with x-ray diffraction (XRD) measurements. These diffraction measurements provided us with a more complete understanding of each phase’s structure. In addition to these primary measurement techniques, Scanning Raman Microscopy and absorption spectroscopy provided us with highly spatially resolved measurements concerning molecular ratios.

2.1 Polarized Optical Microscopy

POM was the primary technique used to identify and observe phases in this study. In POM, the sample is placed between crossed polarizers. The polarization of an electromagnetic (EM) wave is the direction along which the electric field oscillates in the transverse wave. Many sources produce light that is essentially unpolarized. The E field of linearly
polarized light is confined to one spatial dimension. In POM, the white light from the source is linearly polarized along the optical axis of the first polarizer, then interacts with the sample, and is then polarized along the optical axis of the second polarizer, referred to as the analyzer. The transmitted light is then observed. If the sample is isotropic the polarization of the light will be unaffected and no light will be transmitted through the analyzer as seen in Figure 2.1. However, if the sample is anisotropic, like the polarizers themselves, and the optical axis of the sample is not parallel to either the polarizer or analyzer, then light will be transmitted. Materials with this ability are referred to as birefringent, meaning that the index of refraction of the material is dependent upon the polarization of the light. This birefringence is due to the anisotropy of the materials structure, which creates an optical axis, as seen in Figure 2.2. The component of a linearly polarized wave parallel to this optical axis will experience an index of refraction \((n_o)\) that is different than the index for the component perpendicular to the optical axis \((n_e)\). The difference between these indices is the quantitative value termed the birefringence. The phase velocity of light in birefringent media depends on the polarization and frequency due to dispersion. Therefore, the parallel and perpendicular components of an incident wave will have different velocities in the medium.
Figure 2.2: Anisotropic molecules in a birefringent state demonstrating their ability to act as a polarizer with the delocalized electron (e-) able to move along the optical axis. Source: Yamaguchi [3].
When the rays exit the birefringent media, the superposition of the components will produce a new waveform with a different polarization and frequency than the incident beam. This phenomena is responsible for the brilliant colors and contrast in POM images. If the index of refraction and the material’s thickness is known, the color of the texture can be deduced using a Michel-Levy table, shown in Figure 2.3.

![Michel-Levy Chart](image)

Figure 2.3: The color of a material in POM is a function of the thickness of the material and the material’s birefringence. This relationship is summarized in the Michel-Levy Chart. Source: Olympus America Inc.

### 2.2 X-ray Diffraction

X-ray diffraction (XRD) experiments were performed in the SMRC and at the ALS at Lawrence Berkeley National Laboratory. The synchrotron source at the ALS was brighter and allowed for better resolution of scattering peaks. Liquid crystals are different than liquids because they have some form of ordering. In the nematic phase, this is the orientational ordering of the directors. In the columnar phases, this is the orientational order and the 2D positional ordering of the columns. This ordering results in the formation of lattices that demonstrate spatial repetition in electron density. X-ray diffraction is a method used to measure this repetition in electron density. When an x-ray beam collides with the electron
cloud of an atom, the cloud is moved due to the electric force. The movement of the cloud results in the reradiation of the x-ray with the same frequency. When the collision is elastic, it is referred to as Rayleigh scattering. Inelastic collisions will be discussed in Section 2.6. In a lattice, these collisions occur periodically. When these reradiated waves constructively interfere, a peak in the intensity of the reraditated waves is obtained. This constructive interference occurs when the Bragg condition given in Equation 2.1 is satisfied. The variables in this equation are explained in Figure 2.4. Thus by measuring the intensity of the scattered waves at different angles of diffraction (2θ) from the x-ray beam of known wavelength, one can deduce the lattice spacing of a material.

\[ n\lambda = 2d \sin \theta \] (2.1)

The x-ray diffraction systems used in this study had 2D areal detectors. My samples had many small domains of alignment in the beam path, which results in a powder scattering pattern, or a complete circle of radius Q in the areal detector image as seen in Figure 2.5. The radius Q is the magnitude of the reciprocal lattice vector, which is the Fourier transform of the real-space lattice or direct lattice. Conveniently, the reciprocal lattice of a hexagonal columnar lattice (shown in Figure 1.1) is itself a hexagonal columnar lattice. Because the magnitude of the lattice parameters describing the direct lattice in 2D are equal, the reciprocal lattice vectors are equal as well and related to the intercolumn spacing by Equation 2.2. In the dimension parallel to the aggregates, the stacking distance \( h_s \) is related to the wide angle Q value by Equation 2.3

\[ Q = \frac{2\pi}{d_c} \] (2.2)

\[ Q = \frac{2\pi}{h_s} \] (2.3)
Figure 2.4: Reflection of an x-ray beam that satisfies the Bragg condition for constructive interference. $\theta$ is the angle between the incident beam and the plane of atoms. $d$ is the length between identical planes. $n$ is any positive integer. $\lambda$ is the x-ray wavelength.

Figure 2.5: A 2D detector image. The vertical lines are the location of stitching between different shots. $Q$ vectors are measured radially from the center of the beamstop (blue shadow at bottom). The intensity is averaged over the circle corresponding to each $Q$. The bright inner ring is in the small-angle region and corresponds to the intercolumn distance. The outer ring lies in the wide-angle region and corresponds to the stacking height.
2.3 Sunset Yellow

Sunset yellow is an organic molecule used in food dyes, cosmetics, and drugs. Sunset yellow consists of three complete carbon rings and two sodium ions on the periphery. The molecule is chromonic giving it a relatively flat shape with the three rings lying in the same plane as the page in Figure 2.6. The hydrogen in the hydroxyl group may instead be bonded to one of the nitrogens [5]. The three rings are aromatic with delocalized electrons making them hydrophobic. The periphery of the molecules are hydrophillic due to the negatively charged sulfonate groups. This arrangement of a hydrophobic core and hydrophilic peripheral ions in a relatively flat shape results in the spontaneous stacking of these molecules in solution. The molecules non-covalently bond about their hydrophobic cores and stack like coins into rod shaped aggregates of varying length. Edwards simulated this stacking and revealed that the SSY molecules stack head to tail [4], see Figure 2.7.

![Sunset Yellow molecule](image)

Figure 2.6: Sunset Yellow molecule. 3 carbon rings. Sodium ions on the periphery. In plane with page. Source: Edwards [4]

The SSY in this study was purchased from Sigma Aldrich. At high concentrations, where the mass of the SSY solute is 27% the mass of solute and solvent (27%wt), the SSY aggregates transition into an anisotropic nematic phase. Solutions with SSY can exist in isotropic, nematic, and columnar mesophases. The POM textures for these three phases are
Figure 2.7: Simulation of SSY molecules aggregating into column. The chromonic stacking provides a well defined order to the aggregate. Notice the $\pi$ rotation about both axes in the molecular plane between adjacent molecules. Source: Edwards [4]
shown in Figure 2.8. Additionally, sunset yellow is dichroic in the visible spectrum, meaning that white light polarized in the plane of the molecule (perpendicular to the aggregate axis) will have wavelengths from about 400-550 nm absorbed by the delocalized electrons in the aromatic rings, resulting in the lower energy visible spectrum being transmitted, which gives the material its orange/red color. Light polarized perpendicular to the plane of the molecule will not experience the same absorption and therefore has a weaker yellow orange appearance when viewed using POM. By removing the analyzer in POM the dichroism of a material can be observed. The dichroism can be used to determine the orientation of the aggregates in different domains and also to confirm the presence of SSY. This dichroism and the associated aggregate direction is shown in Figure 2.9.

Figure 2.8: Textures of SSY under POM at room temperature. These isotropic, nematic, and columnar textures were at 28%wt., 33%wt., and 43%wt. respectively.

LC phases of pure SSY have been extensively studied. We reviewed Park[5] and Edwards[4] for this work and found general agreement among the phase diagrams despite different units for concentration. Our results presented in Figure 3.13 with concentration units of weight SSY [mg]/ total solution volume [mL], agree best with Park[5] presented in Figure 2.10 with concentration units of [%wt] calculated as weight solute (SSY)/ total solution weight. Our method for calculating the mg/mL concentration is given in Equation 2.4.

\[
Concentration[mg/ml] = \frac{m_{ssy} + m_{dna}}{\rho_{ssy} + \rho_{dna} + \rho_{water}}
\]
Figure 2.9: SSY in a columnar phase with horizontally polarized light. The red rods show the orientation of the aggregates in the intense orange and dull orange domains. The aggregates are not to scale.

Figure 2.10: Phase Diagram for SSY. Source: Park [5]
2.4 DNA

The second material in the mixture was reverse Dickerson Dodecamer (rDD) DNA. This is a DNA strand with 12 nucleobases consisting of Adenine (A), Guanine (G), Thymine (T), and Cytosine (C). The nucleobases are connected by a sugar-phosphate backbone that runs the length of the molecule. This specific sequence is self-complementary, meaning that it can base pair to itself with the 5 end of one strand bonded to the 3 end of the other and vice versa via hydrogen bonding. This allows for the synthesis of a single sequence rather than two unique complementary sequences. When the two single strands bond to form a duplex, the polymer takes the familiar double helix structure of DNA. The pitch of this helix can vary with temperature and concentration, but is commonly cited at around 10.5 base pairs. The mass of rDD is 3646 Daltons. The sequence and associated dimensions are given in Figure 2.11. These duplexes have many similarities to the sunset yellow molecule. The

![Image of reverse Dickerson dodecamer with molecular dimensions and double helix structure.]

Figure 2.11: Reverse Dickerson dodecamer with the associated molecular dimensions. Also shown is the familiar double helix structure with accurate pitch for the dodecamer.

The nucleobases contain aromatic carbon rings that are hydrophobic. The pyrimidine bases, T and C, consist of one diazene. The purine bases, G and A, consist of two heterocyclics. The
sugar-phosphate backbone on the outside of the duplex carries one negative charge on the phosphate group resulting in a -2e charge per base pair. This makes the sugar phosphate backbone hydrophilic. This arrangement promotes the end-to-end stacking of the duplexes into longer aggregates in solution [1]. At high concentrations (22%wt) these stacks form anisotropic liquid crystal phases. This process of aggregation and orientational ordering is shown in Figure 2.12.

![Figure 2.12: Aggregation of rDD into rods and then the alignment of these rods into a nematic phase. Source: Nakata [1]](image)

Nano-DNA can exist in isotropic, chiral nematic, and various columnar textures. rDD has two distinct columnar phases denoted C$_1$ and C$_2$. These phases are birefringent and have distinct textures when observed using POM. The LC textures of rDD are shown in Figure 2.14. The DNA duplex is chiral, specifically right handed chiral. A chiral molecule’s mirror image cannot be superposed on the original molecule. This chirality can manifest itself from the aggregate level to the macroscopic scale. When the aggregates of DNA interact in a nematic phase they stack in a helical pattern with the helical director perpendicular to the director of the aggregates. This chiral nematic phase has an associated pitch length, which is the length it takes the local nematic director to complete a 2π rotation, Figure 2.13.
When the helical director is parallel to the direction of observation, the texture is known as a Grandjean nematic. When the helical director is perpendicular to the direction of observation, a distinct fingerprint like texture is observable. Both of these textures are referred to more generally as chiral nematic (N*). DNA is also dichroic, however, this dichroism appears in the ultraviolet spectrum because the hydrogen bond between the base pair prevents the absorption of longer wavelengths. Both DNA and sunset yellow are lyotropic liquid crystals as opposed to thermotropic, meaning that their phases are dependent upon concentration and temperature.

We synthesized the rDD at the SMRC, using the Caruthers Method [11]. We checked the purity of the DNA by determining the optical densities (OD) per milligram using UV absorbance measurements of the aqueous DNA. It was determined that the DNA had 26.39 OD/mg. For a reference, a batch of DNA that failed to exhibit a nematic phase due to impurities had 22.5 OD/mg. In order to check for impurities and truncates in which a base or multiple bases are left off the sequence during synthesis, we performed mass spectroscopy on the DNA using the MALDI/TOF. The mass spectroscopy showed one prominent peak at 3636 Daltons that was approximately 10 Daltons below the theoretical weight of the strand, 3646 Daltons. The 10 Dalton discrepancy is too small to be attributed to a truncate, so we interpreted this as a systematic error in the measurement. Peaks representing potential impurities could also be resolved, but there were no prominent peaks where a truncate would be expected. Compared to previous attempts at synthesis, this new batch of DNA was relatively pure. The phase diagram for rDD can be found on the front plane of Figure 3.13.
Figure 2.13: The ordering of anisotropic molecules into a chiral nematic (\( N^* \)) phase. In (a) the nematic director is represented by the white arrows. The chiral phase has an associated pitch length shown in b. The molecules shown would be a rod cut normal to planes with the same director extend in the horizontal dimensions. Source: Mitov[10]

Figure 2.14: POM images of the chiral nematic, columnar, and higher concentration columnar textures at 23%wt., 35%wt., and 43%wt. respectively. The isotropic fluid is omitted. There are two distinct columnar phases. These textures were at room temperature.
2.5 DNA SSY Mixtures

The similarities in the chromonic ordering and structure of DNA and SSY inspired curiosity in the behavior of the mixtures. rDD and SSY were mixed in powder form and then water was added to obtain the desired concentration. The convention in this work will be to label the ratio of the species as a mass ratio, \( m_{dna} : m_{ssy} \) and the overall concentration as either a %wt or in mg/mL as described by Equation 2.4. At concentrations above 37%wt, these mixtures become highly viscous and difficult to transfer to cells or capillaries. In some cases this issue was circumvented by preparing the solution at a lower concentration, transferring it to the capillary or cell, and then allowing evaporation to drive the concentration up. These mixtures were observed in either glass capillaries or on microscope slides referred to as cells. The cells were observed using POM. The capillaries were observed using POM and x-ray diffraction. Under POM, the bending of light by the cylindrical capillaries was decreased by submerging the capillaries in mineral oil to decrease the difference in refractive index at the round interface. A schematic for these two containers is provided in Figure 2.15. The capillary was flame sealed once the desired concentration was reached. For cells, the solution was placed on a typical glass slide. A thin cover slip was placed over the solution and mineral oil was placed around the cover slip to slow evaporation. For thinner, more stable cells, the cover slip was fixed to the substrate via a mixture of epoxy and 5 micron spacers. The solution is injected via capillary action followed by mineral oil. A second cover slip is then placed on top of the first and more mineral oil is added.
Figure 2.15: The two primary methods for containing/viewing the mixed phases. The sealed cell was used for POM. The capillary was used for both POM and x-ray scattering. The dimensions are not to scale. Capillaries provided for better concentration control.
2.6 Additional Techniques

In addition to POM and XRD, the LC phases of the rDD-SSY mixtures were probed with scanning Raman microscopy and absorption spectroscopy. The Raman instrument used was the Horiba HR Evolution operated by Eric Ellison (CU-Earth Sciences). The Raman microscope shoots a monochromatic laser at a sample. The photons collide inelastically with the molecules in the sample. Depending on the polarity, vibration, and excitation state of the molecule, the photons energy is either increased or decreased. The possible collision types are shown in Figure 2.16. The scattered photon’s energy is measured by the Raman microscope and the increase or decrease in energy is reported as a Raman Shift with units cm\(^{-1}\), typically used in infrared spectroscopy to denote wavelengths per cm. The Raman microscope then repeats this measurement over a range of incident photon energies, generating a spectrum. The power of the Raman microscope is that it can identify specific molecules with very high spatial resolution (< \(\mu\text{m}^2\)). Different molecules have different polarities, vibrations, and excitation states that act as a fingerprint for them in the form of a Raman spectrum. Sunset yellow has a very distinct Raman spectrum that has been studied before. DNA, unfortunately, has a weak Raman signal that is difficult to resolve in mixtures with sunset yellow. For absorption spectroscopy, we used an Ocean Optics fiber optic microscope attachment and measured the spectrum of light transmitted through a 2\(\mu\text{m}\) thick sample. This transmitted spectrum was subtracted from the spectrum transmitted through the control glass cell to obtain the absorption spectrum.
Figure 2.16: Possible interactions of the incident photons with atoms/molecules measured by the scanning Raman microscope. The up arrows show the absorbed energy, and the down arrows show the reradiated energy. The difference in length is proportional to the Raman shift. Source: B&W Tek.
Chapter 3

Results and Discussion

Early in this work, the LC phases for mixtures of SSY and rDD were observed in a contact cell where pure SSY in an isotropic phase was drawn into the cell via capillary action from one side and pure rDD in a nematic phase was drawn in from the opposite side. This creates a continuous distribution of ratios ranging from SSY with trace rDD to rDD with trace SSY. The POM images of this continuous-ratio texture are shown in Figure 3.1. As water evaporated from this cell, chiral nematic ($N^*$), mixed columnar ($C_m$), and a phase separated columnar texture ($C_s$) developed across the continuum of ratios. This contact cell provided the basis for future exploration of the mixture using POM and XRD.
Figure 3.1: Contact cell with the DNA rich solution on the right side and SSY rich solution on the left. The middle region is isotropic. The solution is nematic on the left and right. The unpolarized image on the bottom shows the presence of SSY (orange).
3.1 Miscible Phases

SSY and rDD are miscible in the isotropic, chiral nematic, and lower concentration, mixed columnar texture \((C_M)\). These phases are shown in Figure 3.2. The nematic phase is cholesteric due to the presence of DNA. The color of these textures varies with the ratio of the species and the thickness of the samples. The isotropic, chiral nematic, and mixed columnar textures are shown for a 2:1 sample.

![Figure 3.2: Isotropic, cholesteric nematic, and mixed columnar textures at 22%wt., 30%wt., and 37%wt. respectively. The ratio is 2:1 (rDD:SSY). These textures were at room temperature.](image)

The diffraction spectra for the miscible phases have two peaks. One at small-angle (larger spacing), corresponding to the intercolumn spacing, and one at wide-angle (smaller spacing), corresponding to the molecular stacking height. The small-angle peak for pure SSY in a columnar phase falls in the range of 0.31-0.33 Å\(^{-1}\) with increasing concentration. The wide-angle peak is at 1.8 Å\(^{-1}\) and does not vary significantly with the concentration. The small-angle peak for pure rDD in a columnar phase falls in the range of 0.23-0.26 Å\(^{-1}\) with increasing concentration. The wide-angle peak is difficult to resolve, meaning that the stacking height varies more for rDD. The peak is centered at about 1.79 Å\(^{-1}\). For mixtures of these molecules in the columnar phase, the small-angle peak falls between the peaks of the pure substances. The stacking peak stays at 1.8 Å\(^{-1}\), with the intensity proportional to the amount of SSY. Therefore, the parameters for the hexagonal columnar lattice are, \(19.7 \text{ Å} < d_c < 26.1 \text{ Å} \), and \(h_s=3.5\text{Å}\). The diffuse nematic peaks for the pure solutions and the
mixture is about 2 Å⁻¹ below the columnar phase peak. The melting of a columnar phase into a nematic phase for a 1:2, 46%wt sample is shown in Figure 3.3. The split peak at the intermediate temperatures shows the coexistence of the columnar and nematic phase.

![Graph](image.png)

Figure 3.3: The small angle peak, indicating intercolumn spacing, as the temperature of the sample increases. This sample was 1:2 and 46%wt. This sample changes from a columnar to a nematic phase as the temperature increases. The peaks shown are Voigt fits of the raw data.

The intercolumn spacing for the mixed columnar phases decrease for increasing ratios of SSY. This is shown in Figure 3.4. To compare these spacings the concentrations were normalized to 40%wt. In the columnar phase, the compression of the phase must occur in the 2D hexagonal plane, meaning that if the concentration changes by a factor $\alpha$ the lattice parameters will change by a factor of $\frac{1}{\sqrt{\alpha}}$. For the columnar peaks in Figure 3.4 normalized at 40%wt, the intercolumn spacing is linear with respect to the weight fraction of SSY. The $R^2$ value for this linear relationship is 0.977. The linear relationship between the mass fraction of SSY and the lattice spacing indicates that the columns are homogeneous and that the SSY does not occupy interstitial sites in the DNA aggregates.
Figure 3.4: The small-angle peak, indicating intercolumn spacing, for different ratios of rDD:SSY. The peaks shown are Voigt fits of the raw data.
3.2 Phase Separation

The mixed columnar texture phase separates as the concentration increases due to evaporation from the cell. This phase separation is visible in the POM textures as small domains that have a different birefringence than the larger mixed columnar domains in the background. This process is shown in Figure 3.5 occurring over a 6-day period. This separated columnar phase ($C_s$) persists until the LC state becomes a crystal. The separation is thermally reversible. The mixing temperature is a function of concentration and ratio of species. Remixing upon heating is shown for a 1:2 sample in Figure 3.6. Upon cooling, the samples reseparate within 2 minutes of cooling below $T_c$.

![Figure 3.5: Time lapse of phase separation of 2:1 mixture. The mixed columnar texture (green) begins phase separating near the edges first. The orange domains are SSY rich in this texture.](image)

The texture of the separated columnar phase varies depending on concentration, the ratio of the molecules, and the preparation technique. Figure 3.7 shows a chiral separation pattern that developed after thermocycling to the remixing temperature. The dark, crossed brushes emanating from the center of each spiral are characteristic of a focal conic, a columnar texture in which the columnar directors align tangentially around the center point defect. This texture shows that the two separated phases have a common columnar director and that the boundary between the phases is parallel to the director in focal conics. The separated domains appear thinner, like stripes, when the ratio of the species shifts towards DNA. This striped texture is shown in Figure 3.8.
Figure 3.6: The transition from 2 phases to 1 phase as the temperature increases from 26°C to 60°C. This weight ratio is 1:2.

Figure 3.7: On the left, the optical axes are arranged radially, creating a focal conic with the crossed polarizer and analyzer. On the right, phase separation in a spiral pattern about a point defect. This texture was 1:2 at room temperature and formed within minutes of a thermocycle to 70°C.
Figure 3.8: Phase separated texture where the separated phase appears in thin dark lines that extend as the concentration increases. This sample was at 2:1 and 26°C.
To explore the composition of the two coexisting phases, we used a scanning Raman microscope to probe 16 points equally spaced along a 30 µm line extending from the center of the focal conic shown in Figure 3.9. We hoped to see differences in the spectra at different points along the line corresponding to the two different phases. The SSY peaks were clearly visible in the spectra and masked the peaks associated with DNA. DNA has a relatively weak Raman spectra. Additionally, the intensity of the SSY peaks didn’t vary significantly or with any trend across the scan. Figure 3.9 shows the average of the 16 individual spectra as well as the pure SSY spectrum. The peak at 1400 cm$^{-1}$ saturated the detector. The resolution of the Raman microscope is well within the width of the separate domains, so the spectra do confirm that SSY is present in both phases.

Figure 3.9: Raman spectrum for pure SSY and the average spectrum for 16 measurements along the 30 µm line in the POM image.

The absorption spectrum of the phase separated columnar texture was measured by Youngwoo Yi [2]. Figure 3.10 shows the location in the two domains where the spectrum was measured. The spectra confirm the presence of SSY in both domains and show that the growing separated region contains relatively more SSY.
To determine the phase diagram and the structure for the separated phase, high concentration samples were prepared in capillaries and shot at the synchrotron. For solutions prepared in capillaries, it was impossible to tell under POM if the columnar phase had separated due to the thickness of the sample. The small-angle region for a separated columnar phase at 1:2, 46%wt is shown in Figure 3.2. The sharp peak at 0.31 Å is in the range of pure SSY columnar, while the more diffuse peak is in the range of the mixed columnar phases. The quality factor and Q value of these peaks indicates that the peak at 0.31 Å corresponds to a nearly homogeneous SSY columnar phase, which has separated from the mixed columnar phase, consisting of both molecules. A separated columnar phase at 1:2, 50%wt was heated to 86°C and shot at about every 5°C. These spectra are shown in Figure 3.12. The two Q values gradually approach each other and become one indistinguishable phase at 86°C. This behavior is plotted in Figure 3.14.
Figure 3.11: The small angle peaks, indicating intercolumn spacing, for a sample 1:2 46%wt. The split peak indicates that there are two distinct phases. The Q value for the sharp peak is in the range of pure SSY columnar phases.

Figure 3.12: Spectra in the small angle regime for a 1:2, 50%wt. sample thermocycled from 40° to 86°. The two distinct peaks merge at an intermediate spacing.
3.3 Phase Diagram

The phases of the rDD-SSY mixture in the temperature-composition plane for four selected ratios were determined using a combination of XRD data and capillary POM. The back and front plane of Figure 3.13 show the phase diagram for pure SSY and DNA, respectively. The phase separated columnar is denoted in orange. The red curve interpolates the phase separated region on the ratio temperature plane. The diagram begs the question of what relationship exists between the C$_2$ phase of rDD and the phase separation that occurs in the mixture. Perhaps, as the rDD enters this higher concentration phase it begins to exclude the SSY aggregates that had previously occupied lattice sites at random in the mixed columnar phase. The structure of the C$_2$ phase is not well understood. This hypothesis will be explored in the future.
Figure 3.13: The phases for the mixtures at 4 selected ratios. The isotropic states are represented by the transparent portion of the 4 planes. The red curve interpolates the phase separated region on the ratio temperature plane.
Using the data from Figure 3.12 we plotted the Q spacing as a function of temperature for the 50%wt sample. This plot is shown in Figure 3.14. For temperatures greater than 60°C, the data was fitted with a third order polynomial given in Equation 3.1 with the independent variable being switched to the Q spacing. This region was chosen because the function $T(Q)$ is smoother above 60°C. Since the aggregates are homogeneous, we can approximate the Q spacing as a weighted average of the pure columnar phases at 50%wt ($Q_{ssy} = 0.335 \text{ Å}^{-1}$, $Q_{rdd} = 0.26 \text{ Å}^{-1}$). This weighted average is given in Equation 3.2 where $c$ is the SSY mass fraction. We can insert Equation 3.2 into Equation 3.1 to obtain the temperature as a function of SSY mass fraction. This function only applies to mixtures ranging from .5 - .95 SSY mass fraction because the original curve was fit to data corresponding to these ratios. A plot of this function is given in Figure 3.15. From this plot we can discern the phase separated domain of the phase diagram. It also gives $T_c = 83^\circ\text{C}$ and $c_o = .78$. This plot corresponds to the red curve drawn on Figure 3.13.

\begin{equation}
T(Q) = 65935 - 650970 \cdot Q + 2.14 \cdot 10^{6} \cdot Q^{2} - 2.34 \cdot 10^{6} \cdot Q^{3} \tag{3.1}
\end{equation}

\begin{equation}
Q(c) = \sqrt{c \cdot 0.335 + (1-c) \cdot 0.26} \tag{3.2}
\end{equation}
Figure 3.14: Boundary between the mixed columnar phase and the binodal phase separation boundary.
Figure 3.15: This plot shows the region where the mixture phase separates and where it is miscible with the associated upper critical solution temperature and the SSY mass fraction where the phase separation is most stable.
Chapter 4

Conclusion

Mixtures of sunset yellow and reverse Dickerson Dodecamer DNA exhibit well-mixed isotropic, chiral nematic, and uniaxial columnar phases. At higher concentrations, excess sunset yellow separates from the mixture and forms a striped texture. This phase separation occurs at higher concentrations and/or lower temperatures than the mixed columnar phase. The boundaries between the phases form parallel to the columnar director. Moreover, the texture of the separated columnar phase depends on the overall concentration and ratio of the two species. The separated region consists of primarily SSY in a hexagonal columnar lattice with trace rDD. The ”DNA rich” regions can contain a variety of ratios of the species, and in some cases have a hexagonal columnar structure. However, the structure of the DNA rich regions may be more similar to the skewed columnar C_2 phase of pure rDD. This will be investigated further. During aggregation, the molecules selectively stack forming homogeneous rods of pure SSY or pure rDD. This demonstrates the selectivity of DNA during LC catalyzed polymerization. The excluded volume is minimized by a hexagonal columnar lattice for the mixture in C_m. At higher concentrations a phase separated hexagonal lattice (shown in Figure 1.3) maximizes entropy. A summary of these phases is provided in Figure 4. Following experiments on the C_m phase, this work will be condensed into a paper for publication.
Figure 4.1: Model of nematic, mixed columnar, and separated textures. The red stacks represent SSY. The blue rods represent DNA.


